METABOLIC AND ENDOCRINE ASPECTS OF
CORONARY DISEASE

by

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OF CORONARY DISEASE

I N T R O D U C T I O N

The name arteriosclerosis was first used by Lobstein (1829-1833) to describe hardening of the arteries. It is a generic term for three distinct lesions, which are probably most satisfactorily defined in pathological terms.

Atherosclerosis

Atherosclerosis is the name first introduced by Marchand (1904) to describe lesions of the arterial intima, which are characterised by focal thickening and the presence of lipid material in and between the cellular elements. At one extreme there may be marked fibrosis and minimal lipid deposition, and at the other there may be little fibrosis and massive lipid deposition and necrosis; it is from this soft yellow pultaceous material that the disease derives the prefix athero (or, in Greek, athere $\alpha\theta\eta\rho\eta$), meaning mush or gruel. The essential atheromatous/

atheromatous lesion is the fatty and fibrous intimal plaque; these plaques are patchy and irregular and occlude the arterial lumen to a greater or lesser degree. Major and moderate sized vessels are involved, particularly the aorta, the coronary, cerebral and mesenteric arteries.

Medial Sclerosis

A different type of arteriosclerosis was described by Mönckeberg (1903). It consists of necrosis and calcification of the media and is not necessarily associated with intimal lesions. Medium sized arteries, such as the arteries of the extremities and the temporal arteries, are most commonly involved; calcification occurs in circular and plate-like masses and cause gross distortion of the arterial wall and the pipe-stem hardening familiar to the clinician.

Diffuse Arteriolar Sclerosis

A third type of arteriosclerosis involves small arteries, particularly of the viscera, 100 microns or less in diameter. The lesions are characterised by subintimal hyaline thickening and elastic hyperplasia, and are particularly marked in the renal arteries in association with hypertension.

It is exclusively with atherosclerosis that thesis is concerned. The pleomorphism of atherosclerotic lesions, which range from the purely fibrous to the fatty necrotic deposit, is so remarkable that it is unlikely that any single aetiological concept will explain their derivation. There have been two fundamentally different approaches to the problem of the aetiology of atherosclerosis: one holds that atherosclerosis is the inevitable result of advancing years, and the other postulates that it is an active and potentially reversible process not exclusively associated with age.

The ageing theory of atherogenesis has received support from the fact that the incidence and extent of atherosclerotic lesions increases with age. Atherosclerosis is regarded as an irreversible process which will eventually over-come us all, provided we survive long enough. Moschowitz (1942), in his monograph on vascular sclerosis, concluded "it is hardly likely that any method of therapy will ever be discovered which will restore diseased vessels to their normal texture. Nor can arteriosclerosis be prevented, no more than grey hair or facial wrinkles." Whereas the histological and physico-chemical/

4.

chemical tissue changes which are associated
with advancing years undoubtedly contribute to
the production of vascular lesions, they do not
wholly explain some of the established facts
concerning atherosclerosis.   Any theory of the
genesis of atherosclerosis must provide plausible
explanations for the following facts, which will
be elaborated during the course of this thesis:
atherosclerotic lesions may be absent in advanced
age and present in childhood, and they are common
in early adult life;   atherosclerotic lesions
involve the arterial tree in an irregular and
patchy distribution;   there is a definite re-
lation between the incidence of atherosclerotic
lesions with sex and with race; and atherosclerotic
lesions can be produced experimentally in various
species by differing chemical and physical agents.
These observations are most satisfactorily explain-
ed by regarding atherosclerosis as an active
disease rather than solely as an incurable con-
sequence of ageing.

  In general, atherosclerosis involves the
abdominal aorta more extensively and earlier than
any other vessel, but slight extrusion of intimal
plaques have little significant effect on the
lumen,/

lumen, whereas similar intimal thickening in
the coronary or cerebral arteries may cause
partial or total occlusion of the lumen.   The
incidence of atherosclerosis in the coronary
arteries is not much less than that of the aorta,
but clearly the consequence of coronary athero-
sclerosis are more disasterous than those of
aortic atherosclerosis.   It is, therefore, the
aetiology of atherosclerosis of the coronary
arteries that is the particular subject of this
thesis.

THE PURPOSE OF THIS THESIS

The purpose of this thesis is twofold.
First, to examine the nature and significance
of certain metabolic abnormalities associated
with ischaemic heart disease.   Second, to
propose and expound a thesis that disturbance
of the physiological balance of the endocrine
glands could contribute to the production of
these metabolic abnormalities and to the develop-
ment of coronary atherosclerosis, and thus be
of major importance in the aetiology of ischaemic
heart disease.

The thesis is divided into four principle
parts.   The first part concerns the development
of atherosclerosis of the coronary arteries, and
the relationship of coronary atherosclerosis to
ischaemic heart disease.   The second part des-
cribes the nature and influences of the circulating
lipids and lipoproteins in health, and the
abnormalities associated with ischaemic heart dis-
ease.   The third part contains the exposition
of the thesis that an endocrine imbalance could
act as an aetiological factor in the development
of/

of ischaemic heart disease.   The fourth part

deals with some of the therapeutic implications

of these metabolic and endocrine aspects of

coronary disease.

PART I

CORONARY ATHEROSCLEROSIS AND ISCHAEMIC HEART DISEASE.

A. ATHEROSCLEROTIC LESIONS.

  1. The early history of hardening of the
      arteries.

        It is now known that aortic atheroma
occurred 3,500 years ago.   Examination of
Egyptian and Coptic mummies, who lived between
1580 B.C. and 525 A.D., revealed extensive patchy
atheroma and calcification in the remains of the
abdominal and thoracic aortae (Ruffer, 1910, 1911);
and Shattock (1909) found calcified atheroma in
the aorta of Menephtah, the Pharaoh of the Exodus.
Unfortunately examination of these mummies gave
no information about the presence of coronary
atheroma as the embalming process always entailed
removal of the heart.   The Egyptians, Greeks and
Romans gave little consideration to cardiac path-
ology, and their knowledge of physiology and
anatomy was mostly speculative.   Thus there was
a gap of many centuries until Bonetus (1678)
described in the Sephulchretum the case of a
fat middle-aged poet who succumbed in a few
minutes after the onset of "distress in breathing":
autopsy revealed ossified coronary arteries, which
were almost, if not completely, occluded and
Bonetus considered that this state of the vessels
was responsible for the symptoms.   Ossification
of the coronary arteries had been reported on a
few occasions before Crell wrote at length in
1740 concerning hardening of the coronary arteries.
He observed/

He observed that the arterial incrustations
were not bony but of a tophaceous nature, and
believed that this induration came from the viscid
juices of the blood.   He considered that these
lesions occurred at any age and were not necess-
arily confined to advanced age.   Von Haller (1755),
in the course of careful dissection, found a
yellow juice between the muscular fibres and the
tunica intima of the arterial wall.   He consid-
ered that this yellow juice gradually hardened
and was the cause of ossification of the arteries.
This accurate localisation of the site of the
atheromatous lesion preceded Virchow's classic
work by a hundred years, but it appears to have
excited little attention and did not receive
confirmation until the following century.
Scarpa (1804) described steatomatous and ulcer-
ative degeneration of the intimal coat of arteries
but thought that it was related to lues venera.
Subsequently there were several reports
incriminating syphilis as a cause of these lesions,
and it was not until 1875 that the first accurate
histological differentiation between the mes-
aortitis of syphilis and the atheromatous
intimal lesion was made by Welch.   Virchow (1856)
emphasised/

emphasised the patchy distribution of athero-
sclerotic lesions; he described in detail the
sites of arterial lesions and considered that
local changes in haemodynamics and mechanical
forces are important in their production.

Evidence that the coronary arteries are
necessary for the maintenance of cardiac function
was first provided by Chirac in 1698 when he tied
the coronary artery of a dog and observed that
soon after the heart had ceased to beat.   In
1809 Burns, in a book published in Edinburgh,
likened the impairment of the heart's function
when the coronary arteries are diseased to the
inability of a limb to continue any vigorous action
if it has had a ligature placed tightly at its
base.   He obviously appreciated the importance
of adequate blood supply for normal function and
believed the blood supply to be impaired by
ossified coronary arteries.   Hodgson (1815)
emphasised the importance of collateral circula-
tion and arteries when the major coronary arteries
are atherosclerotic.   Hope (1832) recognised
that osseous, cartilagenous and steatomatous
degeneration of the coronary arteries is associat-
ed with loss of their elasticity.   Hall (1842)
believed that sudden death was often due to arrest
of the coronary circulation.   He outlined some
investigations/

investigations for testing his hypothesis in
animals and it was confirmed by Erichsen (1842),
who repeated Chirac's original experiment and
ligated the coronary arteries in dogs.   He
recognised that any circumstance that interferes
with the passage of blood through the coronary
arteries, either directly as in ossification of
the coats of those vessels, or indirectly because
of a reduction in ventricular output, may cause
death.

2.   The relation of cholesterol to
      atherosclerotic lesions.

        The name "Cholesterine" was first used
by Chevreul (1816) to describe the crystalline
substance in gallstones.   In 1844 Becquerel and
Rodier stated that there is cholesterol in the
blood and that it increases with advancing years
after the age of 40.   Three years later Vogel
(1847) showed that the steatomatous arterial
degeneration described by Scarpa (1804) is due
to deposits of cholesterol;   he considered that
the presence of these deposits was probably
dependent on an excess of cholesterol in the
blood.   Virchow (1856) also believed that
atheromatous lesions resulted from a primary
imbibition from the blood, and he did not believe
that/

that this was purely passive because an almost
neoplastic proliferation of the intimal connective
tissue preceded any fatty degeneration and
softening. Lipoidal masses in the aorta are
doubly refractile because of the presence of
cholesterol esters (Mettenheimer, 1857), and the
fat content of the aorta increases with the
severity of the atherosclerosis (Gazert, 1899).
Aschoff (1906) emphasised the great abundance
of cholesterol esters in aortic atheroma, and
Windaus (1910) undertook for Aschoff the chemical
analysis of atheromatous aortae and found that
they contain 7 times as much free cholesterol and
20 to 26 times as much ester-cholesterol as
normal aortae.

Whereas the presence of cholesterol in
the atheromatous lesion had been recognised for
a number of years, its significance was not fully
appreciated until Anitschkow and Chalatow (1913)
succeeded in producing a fairly typical picture
of atherosclerosis by feeding rabbits pure
cholesterol dissolved in vegetable oil. Since
these early experiments atherosclerotic lesions,
characterised initially by the presence of fine
fat droplets and foam cells in the extracellular
ground/

ground substance, have been produced repeatedly
in rabbits, dogs and chicks as a result of the
addition of cholesterol to the diet.   Similar
arterial lesions have been produced by the
parenteral administration of cholesterol emulsions
(Pollak, 1949) and the intravenous administration
to normal rabbits of hypercholesterolaemic plasma
from cholesterol-fed rabbits (Bradgon, 1952).
In the human the lipid composition of athero-
sclerotic plaques (Buck and Rossiter, 1951) and
of the plasma is similar enough to suggest that
the lipids of the plaques have their origin in the
plasma (Weinhouse and Hirsch, 1940).   The oral
feeding of tritium-labelled cholesterol to men
has resulted in its demonstration in the plasma
and subsequently in aortic atherosclerotic plaques
(Biggs et al, 1952), and this indicates, therefore,
that exogenous cholesterol enters into the
metabolism of the atherosclerotic human aorta.
In most species the extent and number of cholest-
erol-induced atheroslcerotic lesions is proportion-
al to the degree of hypercholesterolaemia
(Anitschkow, 1933; Katz and Stamler, 1953); this
does not indicate, however, that preceding
hypercholesterolaemia/

hypercholesterolaemia is a sine qua non for the development of typical atheromatous lesions, since such lesions can develop at sites of local anoxia which may favour precipitation of cholesterol in the intima.

3. The relation of blood coagulation to atherosclerotic lesions.

Ideas concerning the formation of fibrin and thrombi were confused and vague until the era of Rokitansky and Virchow. Rokitansky (1841-46) believed that excessive fibrin deposition on the arterial intima leads to intimal and subintimal hypertrophy, atheroma and ossification. Many eminent physicians of the early part of the 19th century considered that fibrin formation and subsequent thrombosis was the result of inflammation of the arterial wall, and Cruveilhier (1849-62) wrote extensively on intravascular clotting and asserted that phlebitis was not only the cause of thrombosis but that it "dominated all pathology". He considered that an arteritis was the foundation of arterial clots, and that the pultaceous matter of atheromatous ulcers was carried by the blood and deposited on arterial walls where inflammation occurred. The subsequent erosions were then repaired by local clotting of the blood. Virchow (1858) was convinced that/

that "inflammation of the blood" was much misunderstood. He studied thrombi and the process of their formation most systematically and stressed the importance of changes in the blood itself, slowing of the circulation and non-inflammatory changes in the vessel wall.

In subsequent years pathologists and physicians were primarily interested in thrombosis as a cause of occlusion of a coronary artery with the resultant acute classical clinical picture. It was not until recently that Duguid (1946, 1949) re-examined Rokitansky's original concept and produced clear-cut evidence that some atherosclerotic lesions result from organisation of fibrin deposits on the intimal surface. Duguid maintains that anoxaemia and fatty degeneration occur deep to the fibrin deposit which becomes covered with endothelium and incorporated into the arterial wall. The degree of narrowing of an atherosclerotic coronary artery is difficult to assess at autopsy for in life the blood pressure tends to iron out excrescences and maintain a smooth intimal lining and full lumen. Indeed, Duguid and Robertson (1955) believe that a vessel which is atherosclerotic is dilated during life and that narrowing of the lumen results/

results from fibrin deposition and thrombosis.
There is little doubt that fibrin deposition
contributes towards the production of arterial
intimal lesions; it is probable, however, that
the lesions which depend primarily on fibrin
deposition are predominantly fibrous in nature.

4.   The coronary circulation and the develop-
     ment of atherosclerotic lesions

          Although the aorta is the vessel in which
atherosclerosis attains its earliest and most
extensive expression (Willius et al, 1933; Albert,
1938), the coronary arteries are far more exten-
sively involved than arteries of corresponding
size.   Certain characteristics of the coronary
circulation may explain this selection.

          The relationship between extravascular
and intravascular pressure may be important in
the development of atherosclerosis.   Coronary
arteries which penetrate the myocardium are rarely
the site of atheroma (Duff and McMillan, 1951;
Geiringer, 1951) probably because the extra-
vascular systolic pressure, produced by the con-
tracting myocardium, is equal to or greater than
the intravascular pressure; thus there is
virtually no passage of lipid material from the
lumen/

lumen into the wall of an artery penetrating
the myocardium. Furthermore, muscular con-
traction has the effect of massaging any small
amount of lipid material from the site of
penetration to a site subject to less violent
extravascular pressure, such as the subepicardial
arteries. In the subepicardial vessels the
lesions are most prominent in the part of the
subintimal region nearest to the myocardium
(Wolkoff, 1929), possibly because of the greater
external movement at this side, but when the
artery lies below a bridge of myocardium, the
buried portion is said to be more uniformly
involved and generally less subject to the disease
(Geiringer, 1951), although this observation has
recently been contested (Edwards et al, 1956).
Subepicardial vessels are commonly the site of
atheromatous deposits and generally such deposits
occur where intravascular pressure is greatest.
When the flow of blood through intramuscular
myocardial arteries is more or less arrested in
systole, the velocity head in the subepicardial
vessels is impeded and the lateral pressure head
is augmented. The resultant high lateral systolic
pressure against the walls of the subepicardial
vessels/

vessels may favour lipid deposition, particularly
in vessels supplying the left ventricle. The
intramural systolic pressure is lower in the
right ventricle and atria, and thus the right
coronary and the circumflex branch of the left
coronary artery are often less involved than the
left anterior descending artery.

There is a definite relationship between
the sites of lipid deposition and areas where
the uniform blood flow is disturbed (Dock, 1950).
The base of the aorta is exposed to rhythmic and
violent vibration and frequently has gross athero-
sclerotic lesions; the anterior cusps of the
mitral valve and the eyelids undergo regular
movement and are common sites for lipid deposition.
When the intramural portions of the myocardial
coronary arteries are more or less occluded during
systole, the blood in the subepicardial portion
of the arteries undergoes turbulence and thus lipid
deposition may be potentiated. Local narrowing,
bifurcations and tortuosity are all associated with
turbulence of the blood. Disturbance of uniform
blood flow can result in instability of the plasma
colloidal equilibrium and could favour cholesterol
deposition (Hueper, 1945).

The/

The intima of the coronary arteries is
developmentally thicker than other arteries and
this thickness increases with age (Wolkoff, 1923;
Gross et al, 1934).   Dock (1946) demonstrated
eccentric intimal thickenings in newborn infants
and showed that the newborn male coronary intima
is $2\frac{1}{2}$ times as thick as that of the newborn
female; he thought that these thickenings were
developmental irregularities which favoured the
subsequent occurrence of atherosclerosis in these
areas, although Fangman and Hellwig (1927) con-
cluded that they were early atherosclerotic
lesions.   These intimal thickenings occur in
varying degree in a large number of newborn
infants of both sexes, and their distribution
resembles that of the adult atherosclerotic lesion
(Minkowski, 1947).

5. <u>Incidence of coronary atherosclerosis.</u>

Coronary atherosclerosis was not described
before the 17th century, yet it is now found
almost universally in adult civilised communities.
It is difficult to determine how much this
represents a true increase in incidence, how much
it is a result of the development of anatomical
and pathological skill and how much it is due to
the/

the increased expectation of life enabling more
people to reach an age when coronary athero-
sclerosis is prevalent.   For instance, Morris
(1951) concluded, from an analysis of the records
of the London Hospital between 1908 and 1949, that
there was no increase in the incidence of coronary
atheroma in routine autopsies during these 42
years.   Although the incidence of atherosclerosis
increases with advancing age (Ophüls, 1926), the
extent and degree of atherosclerotic lesions are
by no means dependent on age.   Typical athero-
sclerotic lesions have been reported from time
to time in children and there is no doubt that
atherosclerosis of the coronary arteries occurs
frequently in young adults.   Studies of the
coronary arteries of battle casualties in World
War I showed atherosclerosis in 11% of youths
between 15 and 20 years of age and 23% of men
between 25 and 30 (Rössle, 1919), and in 51%
of 652 autopsies performed on men between 17
and 45 (Mönckeberg, 1916).   Similarly, Enos
et al (1953) demonstrated well-marked coronary
atherosclerosis in 77% of United States service
men killed in action in Korea, and their average
age was only 22 years.   Conversely, comparatively
minimal/

minimal atherosclerosis was observed in one-third of a series of autopsies in patients dying from all causes over the age of 80 (Groddeck, 1939).

Atherosclerotic lesions are present in the coronary arteries in the majority of the adult inhabitants of the civilised communities of the world. Severe and extensive coronary atherosclerosis was reported in 40% of autopsies performed in men between the ages of 40 and 49, and in 75% of the autopsies in men between the ages of 50 and 59 (White et al, 1950). Blumgart (1951) reported complete occlusion, or marked narrowing of one or more major coronary arteries, in 40% of men who died of non-cardiac causes after the age of 40. These estimates refer to extensive atheromatous lesions of the coronary arteries but Friedberg and Horn (1939) showed that myocardial infarction, necrosis and fibrosis can all occur without gross coronary atherosclerosis and occlusion. Intrusion into the lumen of a major coronary artery by one or two comparatively small, but critically placed, atheromatous deposits may be sufficient to reduce the supply of blood below the demands of the myocardium. Such lesions may act as sites for thrombus formation with further luminal narrowing, and, whether or not/

not thrombosis occurs, a few discrete lesions can lead to myocardial infarction. Thus, it is not only the gross and extensive atherosclerotic lesions which are important.

B.   CLINICAL FEATURES OF CORONARY ATHEROSCLEROSIS

    1.   The early history of chest pain, angina pectoris and coronary thrombosis.

From the time of the Egyptian and Greek civilisations it was thought that the heart was immune from disease.   This concept was handed down over the centuries and was not seriously questioned until the beginning of the 17th century.

    a.   Praecordial pain

William Harvey described, probably for the first time, the association of pain in the chest with myocardial disease.   He recorded in 1649 the case of "a noble knight, Sir Robert Darcy, when he had reached to about the middle period of life, made frequent complaint of a certain oppressive pain in the chest ('dolore quondam pectoris oppressivo') especially in the night season.   The disease became worse and worse and he by and by became cachectic and dropsical, and finally died in one of his severely oppressive paroxysms ('in uno paroxissimo vehementer oppressus').   In his body the wall of the left/

left ventricle of the heart was ruptured, and
there was a hole of such size as to admit any
of my fingers although the ventricular wall
appeared thick and strong". The praecordial
pain of coronary disease was first described by
Morgagni (1769) in his celebrated "De Sedibus
et Causis Morborum". In October 1707 he ex-
amined the body of a woman "the mother of a
family, who was two-and-forty years of age, had
liv'd long in a state of infirm health, and had
long been subject to a kind of paroxysm, which
appeared in the following manner: on using pretty
quick exercise of body, a kind of violent un-
easiness came on, within the upper part of the
thorax, on the left side, join'd with a diffi-
culty in breathing, and a numbness of the left
arm: all of which symptoms soon remitted when
these motions ceased". On a journey to Venice
in October, 1707 she had just such a paroxysm
"and saying that she should die; she actually
died on the spot". Extensive irregular hard-
ening and ossification was found throughout the
entire length of the aorta. Morgagni described
another patient whose symptoms suggested angina
and in whom aortic ossification was found. In
other cases he described atheromatous lesions,
"prominence/

"prominence and pustules", in the coronary
arteries.   Morgagni himself died from a ruptured
ventricle.

    b.   Angina pectoris

        On July 21st 1768, William Heberden read
a paper to the Royal College of Physicians in
London entitled "Some Account of a Disorder of
the Breast".   He began "There is a disorder of
the breast, marked with strong and peculiar
symptoms, considerable for the kind of danger
belonging to it, and not extremely rare, of
which I do not recollect any mention among
medical authors.   The seat of it and sense of
strangling and anxiety with which it is attended,
may make it not improperly be called Angina
Pectoris".   He described the classical features
of angina of effort and also nocturnal attacks
lasting for hours.   He also described the case
of one man who may very well have had a coronary
thrombosis;   "And I have met with one, in whom
it once continued for several days, during all
of which time the patient seemed to be in imminent
danger of death".   Heberden did not recognise
that disease of the coronary arteries was the
primary factor: "The pulse is................
not disturbed by this pain, and consequently the
heart/

heart is not affected by it; which I have had an opportunity of knowing by feeling the pulse, during the paroxysm;"

In 1772 Heberden read a letter which he had received from an unkown sufferer of angina pectoris. He described the subsequent post-mortem which was performed by his friend John Hunter and said "no manifest cause of death could be discovered". In the same year Wall wrote to Hebeden from Worcester and gave an account of the post-mortem examination of a man aged 66, who had had angina. He reported that "the semi-lunar valves, placed at the origin of the aorta, were found to be perfectly ossified". It is inter-esting that the first case of angina, in which an abnormality was found within the heart, was due to calcific aortic stenosis and that in this case the coronary arteries were possibly not particularly diseased. In 1775 John Hunter describes the post-mortem of a man, who had died in a violent transport of anger and who had previously had angina of effort. "The two coronary arteries, from their origin to many of their ramifications upon the heart, were become one piece of bone". In the following year/

year 1776 Edward Jenner saw John Hunter in
Bath, where the latter was convalescing after
his second attack of angina pectoris, and wrote
a most interesting letter to Heberden; he
suggested for the first time the association of
disease of the coronary arteries with angina
pectoris.   He had examined two hearts after
death and "in the first I found no material
disease of the heart, except that the coronary
artery appeared thickened".   In the second he
"found the same appearance of the coronary
arteries as in the former case......... a kind
of firm, fleshy tube formed within the vessels,
with a considerable quantity of ossific material
dispersed irregularly through it.   The importance
of the coronaries, and how much the heart must
suffer from their not being able to perform their
functions (we cannot be surprised at the painful
spasms), is a subject I need not enlarge upon,
therefore I shall just remark that it is possible
that all the symptoms may arise from this one
circumstance...........   Should it be admitted
that this is the cause of the disease, I fear
that the medical world may seek in vain for a
remedy".   When John Hunter died in 1793 (17 years
after/

after Jenner put forward his hypothesis which
was not published for fear that Hunter would
read it) his coronary arteries were found to be
converted into open bony tubes.   By 1802 Heberden
was able to report that he had seen nearly 100
patients with angina pectoris;   the majority
were men near or past 50, but included 3 women
and 1 boy aged 12.

      At the beginning of the 19th century
there were numberous reports of cases of angina
pectoris but disease of the coronary arteries
was by no means established as a cause.   It
was variously described as a nervous affliction
of the heart or as a consequence of syphilis,
and comparatively little attention was given to
the conclusions of Jenner and Parry (1799).
Although there were numerous reports of cases
of angina during the next 100 years, it was by
no means common and it is remarkable that Sir
William Osler (1897) saw a total of only 40 cases
during all his years of clinical experience.
Osler did not see a single case of angina during
the ten years of his private and hospital practice
in Montreal, and he observed that "it is too
narrow a view to suppose the aetiology identical
with that of arteriosclerosis.   The one is so
common/

common and the other comparatively rare".
Sir James Mackenzie, with his early experience
in general practice and later as a leading
cardiologist, wrote as late as 1923 that only
380 patients had consulted him on account of
angina pectoris.

    c.   Coronary thrombosis.

        Thrombosis of a coronary artery was con-
firmed at autopsy, and first diagnosed during
life by Hammer (1876), and the first complete
and accurate description of myocardial infarct-
ion was made in 1880 by Weigert.   Welch (1899)
emphasised that coronary thrombosis occurred
in relation to atherosclerosis of the coronary
arteries.   In 1910 Obrastzow and Straschesko
presented the first complete clinical description
of sudden occlusion of a coronary artery; they
described three patients, two of whom were
diagnosed correctly during life, who had autopsy
evidence of a thrombosis of a coronary artery.
Two years later Herrick (1912) elaborated the
clinical features and indicated for the first
time that sudden occlusion of a coronary artery
is not necessarily fatal.   Despite these reports
it was not until about 1920 that coronary
thrombosis was seriously considered as/

as an antemortem diagnosis.

2. The incidence of ischaemic heart disease.

In 1920 the crude mortality from
ischaemic heart disease (see foot of page) was
22 per million living but in 1955 it was 1575
per million living (Registrar-General's Statis-
tical Tables, England and Wales, 1920-1955);
this represents a seventy-fold increase (Fig. 1).
A series of 3,000 patients suffering from some
form of heart disease was assembled in 1925 and
has recently been contrasted with a comparable
series of 3,000 patients assembled by the same
physician in 1950 (White, 1953): the proportion
of patients identified as suffering from ischae-
mic heart disease was 20% in 1925 and 48.5% in
1950.   In a careful clinical and epidemiological
survey Ryle and Russell (1949) concluded that the
increase in the incidence of ischaemic heart
disease is real and probably not influenced
greatly by changing fashions in death certifi-
cation, such as the transference of cases from
the designations of myocardial and cardiovascular
degeneration to that of diseases of the coronary
arteries and angina.

Ischaemic heart disease is used to describe the
international statistical classification of
arteriosclerotic heart disease (420.0), heart
disease specified as/coronary arteries (420.1)
and angina pectoris without specific mention of
the coronary arteries (420.2).

Fig. 1. The crude annual death rate from ischaemic heart disease per million persons living in England and Wales from 1920 to 1955.
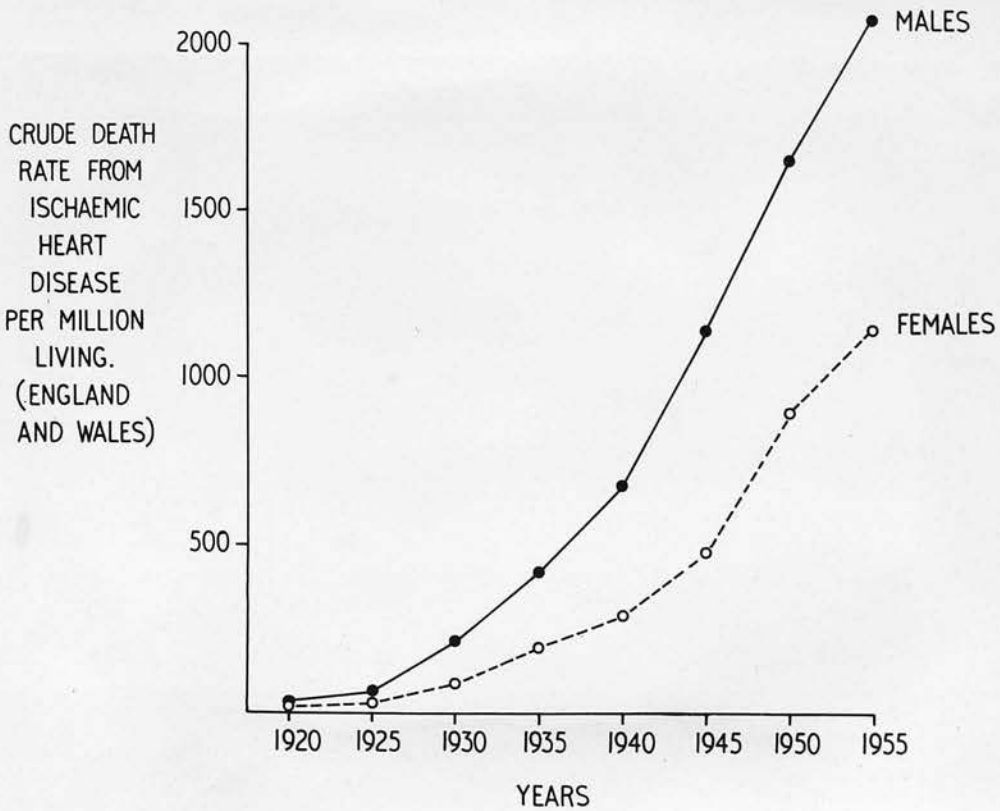
The number of patients admitted annually
with ischaemic heart disease (angina or coronary
thrombosis) to the medical wards of the Edinburgh
Royal Infirmary has been determined and expressed
as a percentage of the total annual medical
admissions for all causes; a similar analysis
has been made for patients with cerebral vascular
disease.   The records allowed analysis over the
last 20 years and the results are shown in Figs.
2 and 3.   Wartime records were not very adequate
and are probably inaccurate on account of the
moving population and the occupation of part of
the hospital by troops.   Although this compara-
tive analysis of morbidity rates is limited by
the total number of available hospital beds, it
suggests that there has been an increase in
morbidity from coronary disease considerably in
excess of the morbidity from cerebral vascular
disease.   A similar trend is seen, particularly
in men, when the mortality from coronary heart
disease and cerebral vascular disease are expressed
as a percentage of the total mortality from all
causes (Registrar-General's Statistical Tables
for Scotland, 1937-1955) (Figs. 4 and 5).

It is hardly conceivable that this in-
crease in the clinical manifestations of coronary
atherosclerosis/

Fig. 2.   The number of patients with ischaemic
           heart disease admitted to the medical
           wards of the Edinburgh Royal Infirmary
           expressed as a percentage of the total
           annual medical admissions for all causes.
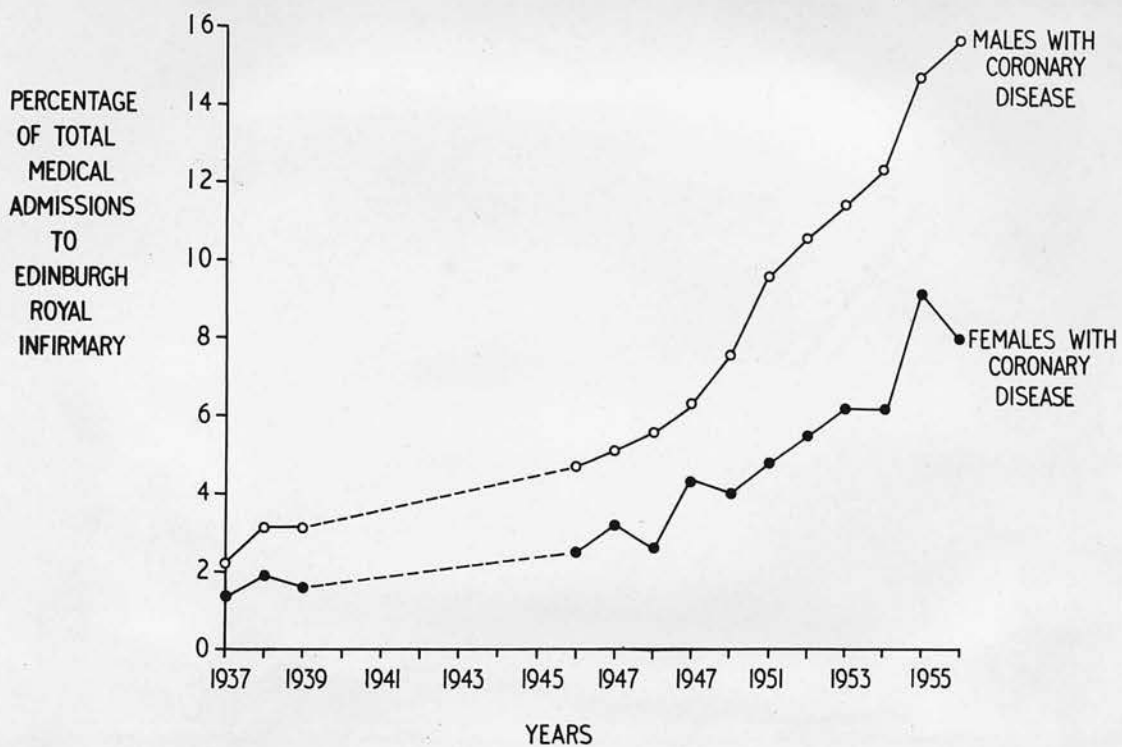
Fig. 3. The number of patients with cerebral
         vascular disease admitted to the medical
         wards of the Edinburgh Royal Infirmary
         expressed as a percentage of the total
         annual medical admissions for all causes.
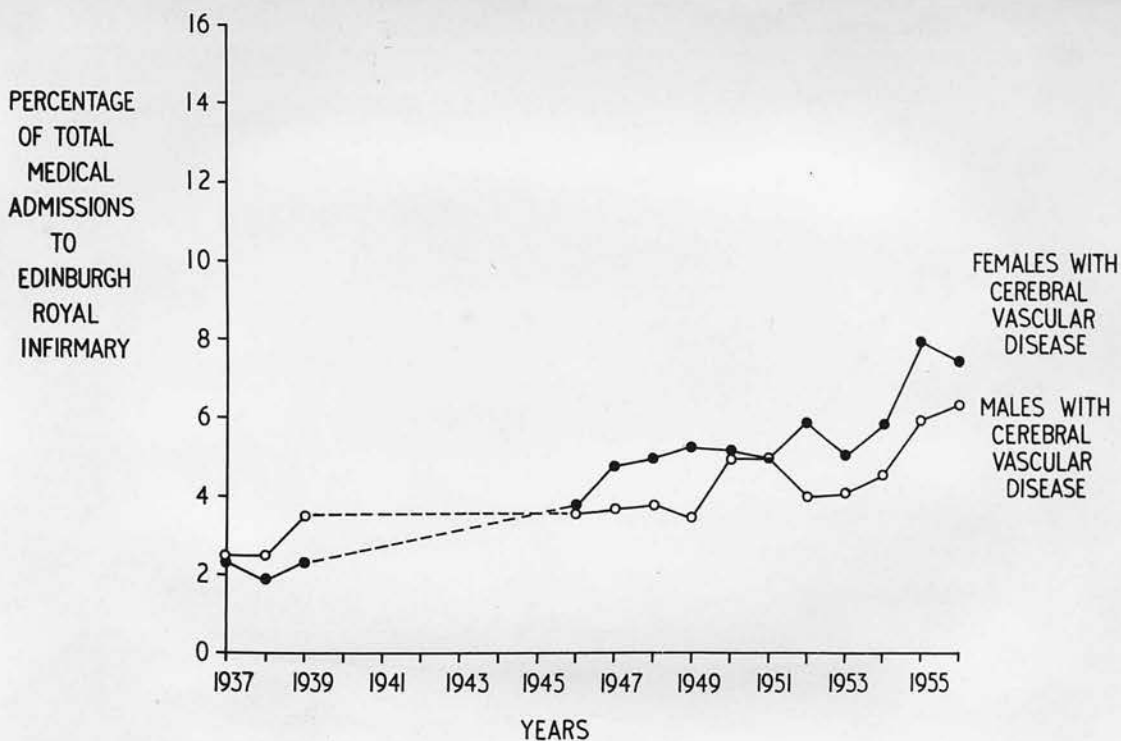
Fig. 4.   The combined male morbidity (R. I. E.)
and mortality (R. G. for Scotland) rates
from ischaemic heart disease and cerebral
vascular disease since 1937;   the broken
lines indicate the war years when records
were unreliable.
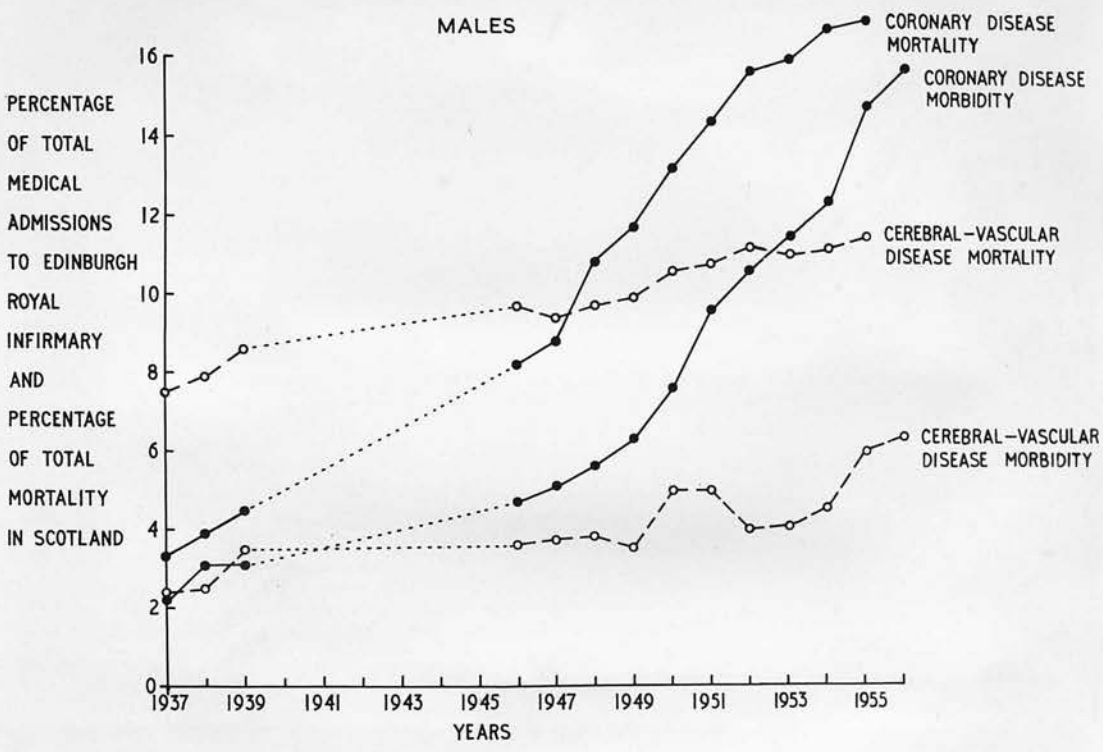
Fig. 5. The combined female morbidity (R. I. E.)
and mortality (R. G. for Scotland) rates
from ischaemic heart disease and cerebral
vascular disease since 1937;   the broken
lines indicate the war years when records
were unreliable.
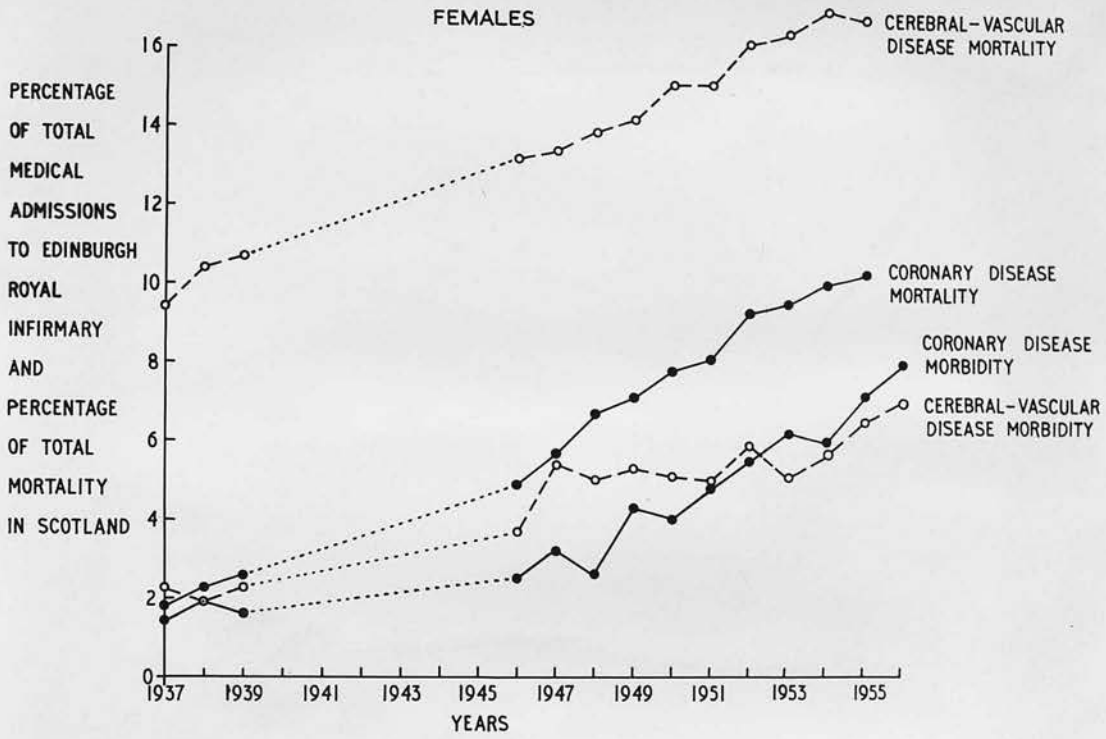
atherosclerosis is entirely due to improved
diagnosis in a population which is living longer,
and it is likely that the increase both in
morbidity and mortality from ischaemic heart
disease represents a real rise in the incidence
of the disease during the last 25 or 30 years.

3. Incidence of coronary thrombosis.

The term coronary thrombosis is most
commonly used to describe the acute clinical
features which accompany myocardial infarction
and does not necessarily indicate whether or not
there has been thrombotic occlusion of a coronary
artery. It has already been indicated that
coronary atherosclerosis may not be increasing
very greatly, yet the incidence of ischaemic
heart disease is rising rapidly. Since
thrombosis of a coronary artery is a cause of
ischaemic heart disease, it is important to assess
its incidence and, in order to do this, it is
necessary to differentiate coronary thrombosis
from myocardial infarction. Myocardial infarc-
tion can occur with and without occlusion of a
coronary artery. Occlusion of a coronary artery
can be due to the formation of a thrombus within
the artery, or due to gradual intrusion into the
lumen by an atherosclerotic plaque; this latter
type/

type of occlusion may not cause any symptoms
during life as its gradual development allows the
formation of adequate collateral vascular channels
(Blumgart et al, 1941).   When there is no
occlusion, infarction can follow prolonged myo-
cardial ischaemia resulting from any discrepancy
between the blood supply and the metabolic demands
of the myocardium.   Friedberg and Horn (1939)
did not observe occlusion in 31% of 91 cases of
myocardial infarction, and by making close trans-
verse sections Master et al (1944) found occlusion
in only 48 of 100 cases of myocardial infarction.

It is probable that only about half the
cases of myocardial infarction have recent and
occlusive thrombosis of a coronary artery.   Yater
et al (1948) examined 450 hearts from United States
service men who died from ischaemic heart disease
between the ages of 18 and 39 during World War II.
All had advanced coronary atherosclerosis and 114
had gross myocardial infarction;   in 8 of these
there was no occlusion of any coronary artery, in
27 (24%) there was atherosclerotic occlusion only,
in 55 (50%) there was thrombotic occlusion only
and in the remaining 27 there was both athero-
sclerotic and thrombotic occlusion.   Of the 336
hearts/

hearts without gross myocardial infarction, there
were 38 without coronary occlusion, 148 (44%)
with atherosclerotic occlusion only, 117 (35%)
with thrombotic occlusion only and in the remaining
33 there was both atherosclerotic and thrombotic
occlusion.   Similarly, Branwood and Montgomery
(1956) demonstrated by the use of a rapid and
efficient slicing technique that thrombotic
occlusion occurred in 21% and that atherosclerotic
occlusion occurred in 18% of 61 cases of recent
myocardial infarction; thus, the majority of
their cases showed no occlusion of a coronary
artery.   In 26 subjects, who had a previous
history of angina and died suddenly, there was
gross coronary atherosclerosis but coronary
occlusion in only eight; in 5 of these occlusion
was due to a recent thrombus, and in the remaining
3 occlusion was due to rupture from softened
atheromatous plaques.   Many of the recent occlus-
ive thrombi appeared to be of more recent origin
than the related myocardial infarct and Branwood
and Montgomery have suggested that some occlusive
thrombi may develop as a terminal event after the
establishment of the myocardial infarct.

        Early studies of coronary occlusion
seldom differentiated thrombotic from atheromatous
occlusion/

occlusion and thus there is no satisfactory measure against which the present incidence of thrombotic occlusion may be contrasted. It is not possible, therefore, to decide whether the increasing incidence of ischaemic heart disease is partly due to an increase in the incidence of coronary thrombosis; all that can be said is that recent occlusive coronary thrombosis probably does not account for much more than half the cases of myocardial infarction.

4. Prevalence of ischaemic heart disease.

With the increase in the incidence of ischaemic heart disease, certain interesting features concerning the prevalence and distribution of coronary disease in the community have become apparent.

a. Age distribution.

An analysis has been made of 1,000 consecutive patients, who were admitted to the medical wards of the Edinburgh Royal Infirmary or the cardiac beds of the Western General Hospital, for treatment of a recent myocardial infarct; none of these patients had had a previous myocardial infarct and all had electro-cardiographic evidence of myocardial damage. The maximum incidence of acute myocardial infarction/

infarction occurred in men between the ages of
50 and 59, and in women ten years later between
the ages of 60 and 69 (Fig. 6 and Table I).

A recent analysis of 7,032 autopsies
over 33 years has suggested that the overall
frequency of deaths from atherosclerotic heart
disease has not changed very greatly, but that
there has been an increase in the incidence under
the age of 50 (Saphir et al, 1956).   In the
course of 5 years 237 patients, who developed
ischaemic heart disease under the age of 50, have
been seen at the Department of Cardiology at
                              but
Edinburgh Royal Infirmary;/ expression of this
figure as a percentage of the total patients does
not give any accurate information of the true age
distribution of the disease since many of these
patients were specially referred to the Department
because of the interest of the staff in coronary
disease.   Furthermore, analysis of the Registrar-
General's statistical reports does not offer any
certain indication that the disease may be
occurring with increasing frequency in younger
age groups as it is not possible to allow for the
increasing awareness of coronary disease by the
medical profession and the whole population.

b./

Fig. 6. The age distribution of 1000 consecutive patients with acute myocardial infarction.

b. Sex distribution.

There is a remarkable difference in the
incidence of clinical coronary disease between
the sexes.   Analysis of the sex distribution
of the 1,000 consecutive admissions, referred to
in the preceding subsection, has emphasised the
male predominance at all ages and the infrequency
of the disease in women during their reproductive
years (Table I) (Oliver & Boyd, 1955c; Mussafia
& Puddu, 1956).

Table I.   Sex and age distribution of 1,000
            consecutive patients with recent
            myocardial infarction.

Age	Numbers of cases		Ratio of Men to Women
	Men	Women	
Younger than 35	19	1	19:1
35 - 39	44	3	15:1
40 - 49	188	26	7:1
50 - 59	256	53	5:1
60 - 69	195	86	2:1
Older than 70	68	61	1:1

This sex difference is striking in civilised
communities, but is much less marked in primitive
communities such as the Bantus (Walker et al,
1956), the Navaho Indians and the negroes in the
U.S.A. (Weiss & Gray, 1954).   It appears that
the sex difference is less evident in populations
which are relatively free from coronary disease.
There/

There is less difference in the incidence of
severe atherosclerotic lesions of the coronary
arteries between the sexes when compared with the
sex difference in ischaemic heart disease.   The
ratio of atherosclerotic lesions between men and
women under the age of 40 is 4:3 (Lober, 1953),
whereas the corresponding ratio for ischaemic
heart disease is in the region of 10:1;   similarly,
there is no significant sex difference with re-
gard to atherosclerotic lesions of the aorta
(Boas and Epstein, 1954).

    c. <u>Heredity</u>.

      There is little doubt that hereditary
factors play a part in the production of
ischaemic heart disease and a family history can
be obtained more often in young patients than in
those who are older (Gertler <u>et</u> <u>al</u>, 1953).
Comparison of the parental histories was made
between 100 patients with ischaemic heart disease
and 100 patients with malignant disease of the
skin, nose and throat - there is no reported sex
limiting influence in the incidence of cancer
in these areas.   The malignant 'control' group
was matched in age and sex to the coronary group.
This analysis (Table II) indicates that a history
of angina or coronary thrombosis is more common
in/

in the parents of patients with ischaemic heart
disease than in the parents of patients with
malignant disease.

Table II.   The incidence of coronary disease
in the parents of patients with
ischaemic heart disease.

	Patients with ischaemic heart disease	Patients with malignant disease
No parental history	76	90
One parent with coronary disease	21	10
Both parents with coronary disease	3	0
	100	100

A family history of ischaemic heart
disease probably depends partly on the associated
hypercholesterolaemia which occurs more often
and to a greater extent in young subjects of
coronary disease (see page 76);   an example of
this association of hypercholesterolaemia with
a family history of ischaemic heart disease is
shown in Fig. 7 .   Hypercholesterolaemia is often
inherited (Adlersberg et al, 1949) but its mode
of inheritance is not yet understood.

d.   Body build./

An example of ischaemic heart disease
and hypercholesterolaemia in a family.
The figures represent the plasma cholest-
erol levels in mg%.

d. Body build.

Body build is largely determined by
hereditary influences (Sheldon et al, 1940).
Coronary thrombosis has been reported to be more
common in square muscular men tending to fatness
(endomorphic mesomorphs) (Phipps, 1936; Gertler
et al, 1953). An analysis was made of the
somatotypes of 200 consecutive subjects (149 men
and 51 women) with symptoms of coronary disease;
there was electrocardiographic confirmation of
myocardial infarction in 170 and of ischaemia
before or after the Master two-step test in 30.
This group was contrasted with 200 miscellaneous
inpatients who had no history or clinical features
of cardiac, renal, hepatic, metabolic or wasting
disease; this control group was matched to the
coronary group by age and sex. The height:weight
ratio, or ponderal index, which is regarded as
a useful measure of massiveness, was assessed in
these 400 patients (Ponderal Index $= \dfrac{\text{Ht in cms.}}{\sqrt[3]{\text{Wt in Kgms.}}}$
(Sheldon et al, 1940).   The results are ex-
pressed in Table III.   In men with ischaemic
heart disease over the age of 40 the ponderal
indices differed significantly from the men of
corresponding decades in the control group
$(P = < 0.05)$, but no significant difference was
observed in women. These lower values in men
with/

Table III. The ponderal indices in 200 patients with ischaemic heart disease and 200 controls. (A ponderal index of <39 indicates endomorphy; 39-43 indicates mesomorphy; >43 indicates ectomorphy).

MEN

AGE GROUPS	NUMBERS	MEAN AGE	CONTROL GROUP		CORONARY DISEASE GROUP	
			Ponderal Index	Standard Deviation	Ponderal Index	Standard Deviation
30 - 39	12	36	42.6	±1.6	42.5	±1.1
40 - 49	36	45	42.8	±1.9	41.5	±1.8
50 - 59	52	54	42.7	±2.2	41.8	±1.8
60 - 69	37	64	42.9	±2.1	41.2	±0.3
70+	11	75	43.0	±1.3	40.9	±1.3

WOMEN

AGE GROUPS	NUMBERS	MEAN AGE	CONTROL GROUP		CORONARY DISEASE GROUP	
			Ponderal Index	Standard Deviation	Ponderal Index	Standard Deviation
30 - 49	10	42	41.8	±1.5	41.5	±1.5
50 - 59	12	54	41.2	±2.8	39.6	±2.7
60 - 69	18	64	42.6	±2.4	40.5	±2.6
70+	11	75	41.6	±1.8	40.1	±2.8

with coronary disease confirm that ischaemic heart disease occurs more commonly in endomorphic mesomorphs than in ectomorphs: mesomorphy is the component of masculinity, bone mass and angularity of outline and contour; ectomorphy is the component of linearity and elongation, while endomorphy is the component of softness and roundness but not necessarily of fat.

     e.   Prevalence in disease with abnormal
            lipid metabolism.

        Ischaemic heart disease and severe coronary atherosclerosis both occur commonly and prematurely in association with certain diseases in which there is disturbance of lipid metabolism and a hypercholesterolaemic tendency. In diabetes mellitus the incidence of coronary thrombosis and of coronary atherosclerosis is more than twice normal (see Part III Section B) and atherosclerosis has been regarded as almost universal in diabetes (Barach, 1949). Pronounced hyperlipaemia and hypercholesterolaemia are commonly found in association with diabetes (see Part III, Section C).

        Similarly in hypothyroidism the incidence and severity of ischaemic heart disease and coronary atherosclerosis is increased (see Part III Section B) and there is hypercholesterolaemia (see/

(see Part III, Section C).   In chronic renal
disease and the nephrotic syndrome there is a
significant increase in atherosclerosis (Steiner
& Domanski, 1942) and there is often gross
hypercholesterolaemia (Peters & Man, 1943c).
In familial hypercholesterolaemic xanthomatosis,
the incidence of coronary atherosclerosis, which
is greatly increased, directly parallels the
degree of hypercholesterolaemia and occurs
prematurely (Adlersberg et al, 1949).

C.   CONCLUSION.

Atherosclerotic arterial lesions occurred
thousands of years ago and there is no clear
evidence that their incidence is increasing;
the nature of the coronary circulation predisposes
it to the development of atherosclerotic lesions
which are present in the vast majority of the ad-
ult members of the civilised communities of the
world.   In contrast, ischaemic heart disease,
which was uncommon before this century, accounted
for 20% of all the deaths in Britain in 1955
(Registrar-General's Statistical Tables, England
and Wales, and Scotland, 1955).   There is a
striking male predominance, particularly under
the age of 50, in the incidence of ischaemic
heart disease but this does not apply to the
incidence/

incidence of atherosclerotic lesions of the coronary arteries.

It would seem reasonable to conclude from both these observations that some aetiological factors influence the development of ischaemic heart disease without producing any marked change in the incidence of coronary atherosclerosis.   It might be suggested that thrombosis is such a factor but at present there is no convincing evidence that coronary thrombosis is on the increase, and there is little doubt that thrombosis can become incorporated into the arterial wall;   thus any increase in the incidence of thrombosis would probably be reflected by an increase in atheroma as well. Myocardial infarction rarely occurs spontaneously in any species other than the human and cannot be produced experimentally without traumatic obstruction of a coronary artery.   Yet atherosclerosis occurs spontaneously in a number of species, such as the rabbit and fowl, and can be produced experimentally in others by a variety of constraints, of which cholesterol feeding is almost always one: the extent and degree of these/

these atherosclerotic lesions are more or less
directly proportional to the hypercholesterolaemia
which is induced.   Although in animals myocard-
ial infarction and fibrosis do not occur and
atheroma of the coronary arteries is often
histologically different from the human lesion,
information gained from animal investigations
should be studied in the human.   Since cholest-
erol feeding is almost always necessary in order
to produce coronary atherosclerosis in animals,
cholesterol metabolism and particularly the
circulating cholesterol requires close examin-
ation in relation to coronary atherosclerosis
and ischaemic heart disease in the human.

PART II

THE CIRCULATING LIPIDS AND LIPOPROTEINS IN HEALTH AND IN ISCHAEMIC HEART DISEASE.

Before any consideration of the circulating lipids in relation to coronary disease, their nature and physiological levels will be summarised.

A.   THE CIRCULATING LIPIDS AND LIPOPROTEINS
        IN HEALTH.

    1.   Definition of the circulating lipids.

    a.   Neutral Fat.   These are triglycerides of various saturated and unsaturated fatty acids containing even numbers of carbon atoms. Chylomicrons (Gage and Fish, 1924) are aggregates of neutral fat molecules, which are visible in plasma since they measure approximately 1 micron in diameter, and are largely derived from chyle; they can be used as a measure of fat absorption.

    b.   Fatty Acids.   Neutral fats, esterified cholesterol and the compound lipids all contain fatty acids.   Fatty acids also circulate in a chemically "free" form although physically bound to certain proteins.

        i.   Non-essential fatty acids are mostly saturated and are synthesised from "active acetate" derived from fat, protein and carbohydrate; examples are palmitic (C_{16}) and stearic acid (C_{18}).

        ii.   Essential fatty acids are unsaturated and/

and are not synthesised by the body but must be obtained from the diet. They are principally linoleic, linolenic and arachidonic acids which contain 2, 3 & 4 double bonds respectively. Of the three main circulating lipids, neutral fat, cholesterol and phospholipids (lecithin), the fatty acids of cholesterol esters are the most unsaturated (iodine number = 158: Bloor et al, 1938) and are probably largely of the essential category.

c. Sterols. Cholesterol makes up between 95% and 98% of the circulating sterols but there are also minute quantities of dihydrocholesterol and 7-dehydrocholesterol in circulation. Cholesterol occurs in the plasma in two forms: approximately 30% as "free" cholesterol, when the hydroxyl group is unesterified and therefore can be precipitated by digitonin, and approximately 70% as ester cholesterol when it is esterified with unsaturated fatty acids. Normally circulating cholesterol is always bound to protein whether free or esterified. Sperry (1936) has indicated that the ratio of ester to total cholesterol is remarkably constant.

d. Compound Lipids. Many of these contain phosphorus and all include fatty acids in their structure./

structure.   Phospholipids or phosphatides are
usually classified into lecithins, cephalin and
sphingomyelin.   Lecithins contain the base
choline and make up approximately 80% of the plas-
ma phospholipids.

2.   Definition of the circulating lipoproteins.

For many years the clarity of serum and
plasma suggested that its lipid content is
stabilised in solution by linkage to protein, and
in 1929 Macheboeuf indicated the presence of
lipid-protein complexes by his isolation from
serum of a substance which contained a repro-
ducible proportion of nitrogen and lipids.   It
is now generally believed that neutral fat,
cholesterol (free and esterified forms) and
phospholipids are transported in the circulation
combined with one another and with protein in
macromolecular complexes called lipoproteins.

The properties and structure of the
lipoproteins, and the enzyme systems involved in
their interaction, have not yet been defined but
they are known to differ in chemical constitution,
in solubility, in molecular size and in iso-
electric point.   Cholesterol and phospholipids
are associated with all the protein fractions but
by/

by far the greatest concentration is attached to
the α_1 and β_1 globulin fractions (Blix et al,
1941), and these two lipid-laden globulins are
termed the α and β lipoproteins.

Zone electrophoresis (Wieland and Fischer,
1948), which is based on the different rates of
migration of the protein fractions in a buffer
in an electric field, permits isolation of each
protein component of the serum; actual chemical
analysis of the serum lipoproteins can be ob-
tained by subsequent elution of cholesterol and
phospholipids from the adsorbent which can be
starch or more commonly filter-paper (Nikkilä,
1953; Boyd, 1954). Low temperature ethanol
fractionation separates lipoproteins according
to their physicochemical properties (Russ et al,
1951). The ultracentrifuge produces a force
many thousand times that of gravity and large
molecules, such as lipoproteins, are induced to
undergo flotation or sedimentation according to
the relative density of the molecules and the
medium. The concentration of these lipoproteins
can be determined during ultracentrifugation
by a photographic procedure (Gofman et al, 1949;
de Lalla & Gofman, 1954); in general, the low
density/

density lipoproteins correspond to the β-lipo-proteins and the high density lipoproteins to the α-lipoproteins.

3. The cholesterol:phospholipid (C/P) ratio.

The ratio of the circulating cholesterol to the circulating phospholipids is reasonably constant in health (Peters and Man, 1943a) and there is evidence to indicate that the phospho-lpids have a stabilising effect on the colloidal dispersion of cholesterol (Boyd, 1937). The degree of visible lipaemia appears to be in-versely related to the lecithin content of the serum (Ahrens and Kunkel, 1949), and thus the translucency of the serum is dependent not only on the lipid-protein attachments but also on the ratio of cholesterol to phospholipid within the macromolecule; thus elevation of the C/P ratio is said to favour cholesterol deposition (Ladd et al, 1949). The average cholesterol:phospho-lipid ratio (C/P ratio) of whole plasma is approximately 0.80 in health (see Table VI) the C/P ratio of the β-lipoproteins (low density lipoproteins) is approximately 1.25 and the C/P ratio of the α-lipoproteins (high density lipo-proteins) is approximately 0.55. Thus it is apparent/

apparent that the C/P ratio of whole plasma is
derived from the C/P ratio of the two major
groups of lipoproteins and is dependent on the
ratio of one lipoprotein to the other.

4. Origin and fate of the circulating cholesterol.

The plasma cholesterol is derived from
an exogenous (dietary) and an endogenous
(synthetic) source.   Dietary cholesterol is
absorbed, 70% in an esterified form and 30% in
a "free" (or non-esterified) form, and contributes
directly to the plasma cholesterol.   Isotope
techniques have shown that cholesterol is
synthesised from acetate (Bloch & Rittenberg,
1942) and that all twenty-seven carbon atoms are
derived from acetate (Wuersch, et al, 1952;
Cornforth, et al, 1953).   Moreover, acetyl
coenzyme A (active acetate) has been shown to be
involved in cholesterol biosynthesis (Klein &
Lipman 1953;   Boyd, 1953) and is a common key
intermediate in the metabolic pathways of carbo-
hydrate, protein and fat.   Thus, the three chief
dietary elements, which are all sources of active
acetate, contribute to the endogenous synthesis
of cholesterol.

All/

All animal cells contain cholesterol and tissue slice techniques have shown that many tissues, with the notable exception of brain (Srere et al, 1950), are capable of cholesterol biosynthesis.   However, the liver is the only significant source of plasma cholesterol and there is evidence that in some species the testes, spleen, kidney, lung and adrenals, though possessing the mechanism for cholesterol biosynthesis, in fact withdraw and utilise cholesterol from the plasma under physiological conditions (Landon & Greenberg, 1954).   Many major plasma constituents including glucose, albumin, fibrinogen and probably & globulins, are now known to originate entirely in the liver, and the plasma and liver can be regarded as different compartments of the same system (Gould, 1951);   isotope techniques have indicated that there is a free interchange of cholesterol and of phospholipids between the plasma and the liver (Hahn and Hevesy, 1940; Gould et al, 1951).

Liver cholesterol is excreted into the bile as cholesterol and bile salts (glycocholate), and intestinal cholesterol is partially re-absorbed; biliary excretion and intestinal re-absorption of cholesterol/

cholesterol are important homeostatic mechan-
isms for the maintenance of a constant level
plasma cholesterol.   The transference of plasma
cholesterol to venous extrahepatic tissues, where
the sterol sometimes may be modified into steroid
hormones, is probably largely irreversible.

5.   Normal levels of the circulating lipids
and lipoproteins and the influence of age
and sex.

In all the investigations to be reported
thd plasma total and free cholesterol, hence
esterified cholesterol by difference, has been
estimated by the Sperry-Schoenheimer digitonin
precipitation procedure as modified by Sperry
and Webb (1950); the error of this method has
been $\pm$ 6%.   The phosphorus content of a lipid
extract of plasma has been determined by the
molybdenum blue method of Allen (1940);   the
error of this method has been $\pm$ 5%.   The α and β
lipoproteins of serum have been separated by a
filter-paper zone electrophoresis micro-technique
(Boyd, 1954) and followed by serial cholesterol
estimations.   It has been possible, therefore,
to determine the precise quantity of cholesterol
in mg% attached to the two major lipoprotein
fractions, but for convenience the distribution
of cholesterol between the α and β lipoproteins
has/

has often been expressed as a percentage of the total cholesterol - thus 30% on the α-lipoprotein fraction and 70% on the β-lipoprotein fraction, or an $\alpha : \beta$ lipoprotein ratio of 30:70; the accuracy of this ratio has been $\pm$ 3%. The normal daily intake of food had no immediate effect on the plasma cholesterol and C/P ratio (Table IV) and thus blood samples have not been taken exclusively in the fasting state. There is a slight and inconstant variation in plasma cholesterol during the 24 hours of the day (Boyd, 1935), but in most, if not in all, healthy persons the plasma cholesterol is maintained at each individual's constitutional level and large deviations do not ordinarily occur (Sperry, 1937).

Table IV.   The effect of ordinary meals on the circulating cholesterol and C/P ratio of 12 healthy men.

	Fasting (8.0 am)	3 hours after breakfast (11.30 a.m)	3 hours after lunch (4.0 p.m)
Plasma cholesterol (mg%)	184	187	191
C/P ratio	0.76	0.78	0.78

a.   Infants./

a. <u>Infants</u>.

The plasma cholesterol of newborn
infants is low (Sperry, 1936; Boyd, 1936) but
rises rapidly within the first ten days of life;
analysis of the blood from the umbilical cord of
5 male and 5 female infants is shown in Table V.
From the age of one year there is no significant
increase in plasma cholesterol until puberty
(Kornerup, 1950; Adlersberg <u>et al</u>, 1956) and
after puberty subsequent changes depend on sex.

<u>Table V</u>. The mean circulating lipids and
lipoproteins of 5 male and 5 female
infants at birth (umbilical cord blood)

	Males	Females
Plasma cholesterol (mg%)	55	56
C/P ratio	0.48	0.51
$\alpha : \beta$ lipoprotein ratio	35:65	37:63

b. <u>Men</u>.

The circulating lipids have been studied
in 200 men of all decades from puberty (Table VI)
and the $\alpha : \beta$ lipoprotein ratio has been deter-
mined in 75 of these subjects (Table VII).

Table VI. The mean plasma cholesterol and C/P ratio in 200 apparently healthy men

Decades	Numbers	Mean Age	Plasma Cholesterol in mg.%	Standard Deviation	Range of Plasma Cholesterol	C/P Ratio	Standard Deviation	Range of C/P Ratio
15 – 19	8	18	142	-	104-199	0.75	-	0.41-0.88
20 – 29	24	24	166	± 28	111-208	0.81	± 0.12	0.46-0.92
30 – 39	24	36	170	± 24	112-226	0.80	± 0.15	0.48-0.98
40 – 49	40	45	180	± 33	110-248	0.84	± 0.15	0.52-1.16
50 – 59	52	54	168	± 30	106-230	0.82	± 0.10	0.53-1.07
60 – 69	40	64	168	± 42	100-284	0.80	± 0.14	0.51-1.30
70 +	12	75	171	± 34	124-242	0.77	± 0.12	0.54-0.90

Table VII.   The mean $\alpha : \beta$ lipoprotein ratio
in 75 apparently healthy men.

Decades	Numbers	$\alpha : \beta$ lipoprotein Ratio	Standard Deviation
20 - 29	10	29 : 71	$\pm$ 7.3
30 - 39	16	26 : 74	$\pm$ 7.2
40 - 49	21	23 : 77	$\pm$ 6.8
50 - 59	17	20 : 80	$\pm$ 7.4
60 - 69	8	19 : 81	-
70 +	3	22 : 78	-

The subjects of this study were healthy members
of the University and hospital staffs or in-
patients without evidence of cardiac, renal,
hepatic or metabolic disease.   The plasma
cholesterol and C/P ratio increased from puberty
until the age of 30-35 years, but thereafter no
further significant increase was observed. (Oliver
& Boyd, 1953b).   Most investigators agree
that there is a gradual rise in plasma cholesterol
with increasing age up until 35 years, but there-
after the pattern is less clear.   The results
shown in Table VI indicate that the plasma
cholesterol does not increase significantly in
men   over 35 in Britain and confirm similar
observations in the United States (Page et al,
1935/

1935; Foldes and Murphy, 1946): recently these results have been confirmed by Adlersberg et al, 1956 and Little et al, 1956. Other investigators have shown an increase in plasma cholesterol with increasing age (Melka, 1927; Keys, 1949; Gertler et al, 1950; Keys et al, 1950; Kornerup, 1950; Russ et al, 1951). However, Keys et al (1950), who observed a steady progression with advancing age in a large series of Minnesotan bank clerks did not observe any rise in a group of Neapolitans (Keys et al, 1952), and suggested that the difference between the two studies might be related to the lower fat intake of the Italians. Apparently inconsistent results may depend partly on racial and environmental influences (see section on race page 65) since many of these studies were conducted in different countries. At all events the rise in cholesterol is not very marked and probably not an obligatory con-comitant of ageing.

  c. Women.

       In view of the striking sex difference in the incidence of clinical coronary disease (Page 37), and the relative immunity from coronary disease enjoyed by premenopausal women,
a/

a special study of the circulating lipids and
lipoproteins was made in relation to the two
physiological activities which occur in women
during their reproductive years - the menstrual
cycle and pregnancy.

i.   The Menstrual Cycle.

There have been very few studies of
the circulating lipids in relation to the menstrual
cycle and none in which blood samples have been
obtained at frequent intervals throughout the
cycle.   Elevation of the plasma cholesterol
immediately before menstruation was reported by
Gonalons (1916), by Moynihan (1925) and by Okey
and Boyden (1927) who also observed a rise after
menstruation.

The plasma cholesterol and C/P ratio
were determined in 12 young healthy women, of
whom one was amrried and all were nulliparous,
and also in 12 young healthy men;   these subjects
were students, secretaries or laboratory technic-
ians.   The mean age of the female group was 22
years and the mean age of the male group was 21
years.

The subjects were instructed in the use
of/

of a clinical thermometer, and asked to record their waking oral temperature every morning while still lying in bed.   Ovulation was taken as occurring at or just before the point where the temperature begins to rise during the intermenstrual phase (Rubenstein, 1937).   Blood samples were taken in the fasting state twice weekly over 5 weeks.   It was particularly important to eliminate possible day to day variations in the quantitative analyses, which were therefore performed by making a lipid extract of the plasma immediately after the sample was taken and storing this extract in a sealed glass ampoule in a cool, dark box.   When ten samples had been obtained from each individual, all the estimations were made on the same day.

Definite cyclical changes were observed in all 12 women (Fig. 8) (Oliver & Boyd, 1953a). At ovulation there occurred a striking fall in plasma total cholesterol, due entirely to a fall in plasma ester cholesterol, with a rather less marked fall in plasma phospholipids and therefore a fall in the C/P ratio.   Whereas in the follicular and luteal phases of the cycle there was a greater increase in plasma total cholesterol than/

Fig. 8. The mean changes in the circulating lipids
         of 12 healthy young women during the
         menstrual cycle.   The changes in the
         plasma total and ester cholesterol and in
         the plasma phospholipids are shown as the
         percentage deviation from the mean of the
         entire cycle;   the actual values of the
         C/P ratio are given.
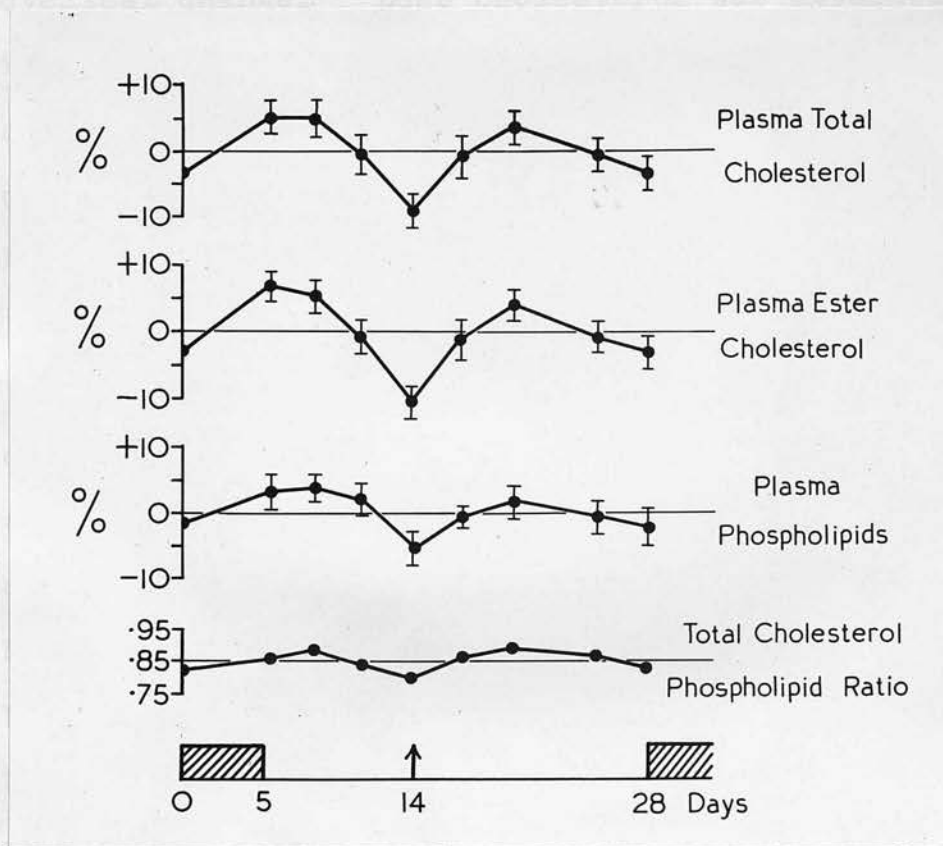
than in plasma phospholipids, and therefore a relative increase in the C/P ratio: Fig. 9 shows the changes which occurred in a typical young woman.   In contrast, there was no regular fluctuation in the circulating lipids in the 12 young healthy men (Fig. 10).

A further group of 12 normal young women were studied and it was found that the distribution of cholesterol between the $\alpha : \beta$ lipoprotein fractions also underwent regular cyclical change.   Less cholesterol was attached to the β-lipoprotein fraction, and thus there was a higher $\alpha : \beta$ ratio at ovulation than at any time during the menstrual cycle (Fig. 11) (Oliver and Boyd, 1955c).

ii.   Pregnancy.

The presence of hypercholesterolaemia during pregnancy was first suggested by Becquerel and Rodier in 1845 and first observed by Neumann and Hermann (1911) and by Chauffard et al, (1911). Early studies, which were all made at or just before term, resulted in discordant views on the behaviour of the circulating lipids in advanced pregnancy.   Gardner and Gainsborough (1929), who were the first to study the lipids of pregnancy with/

Fig. 9.   The changes in the circulating lipids of
           a typical healthy young woman during the
           menstrual cycle.
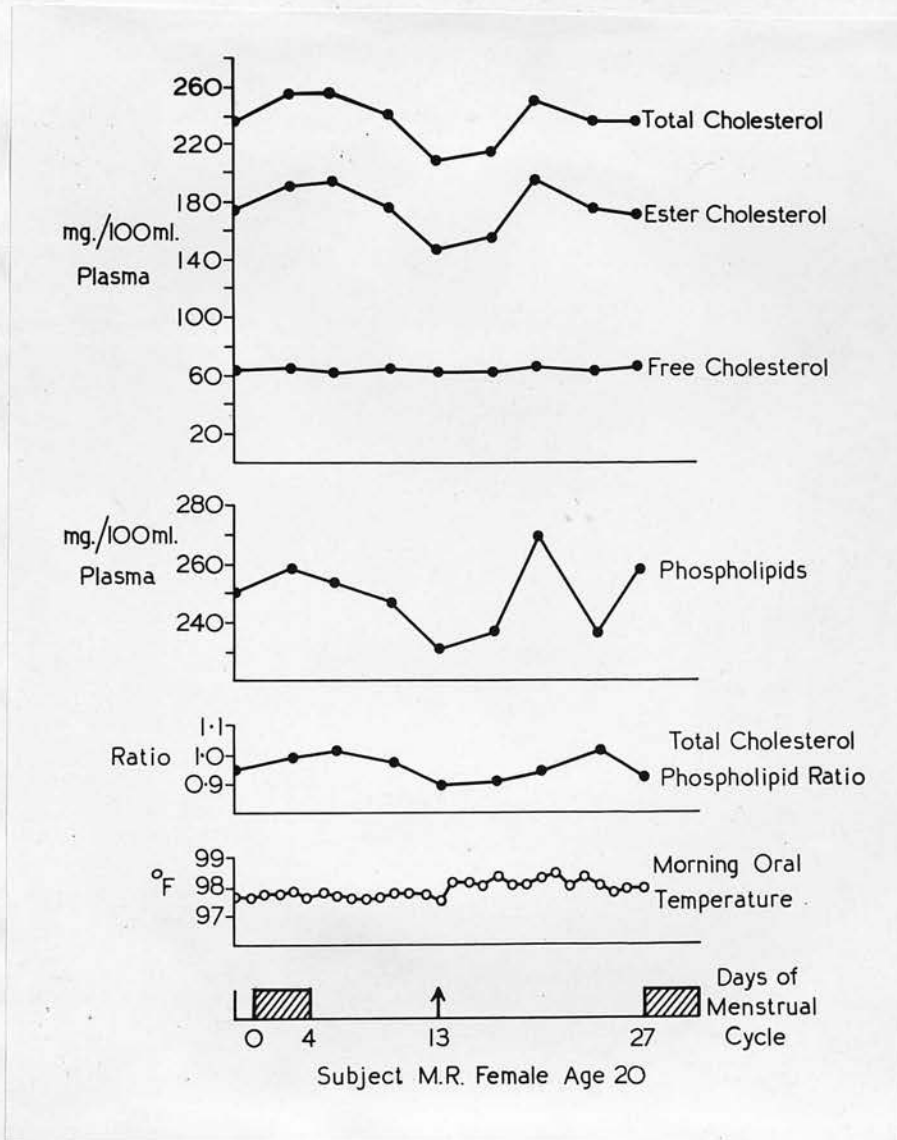
Fig. 10.   The mean changes in the circulating
            lipids of 12 healthy young men over a
            period of 35 days.   The changes in the
            plasma total and ester cholesterol and
            in the plasma phospholipids are shown as
            the percentage deviation from the mean
            of the period of study;   the actual
            values of the C/P ratio are given.
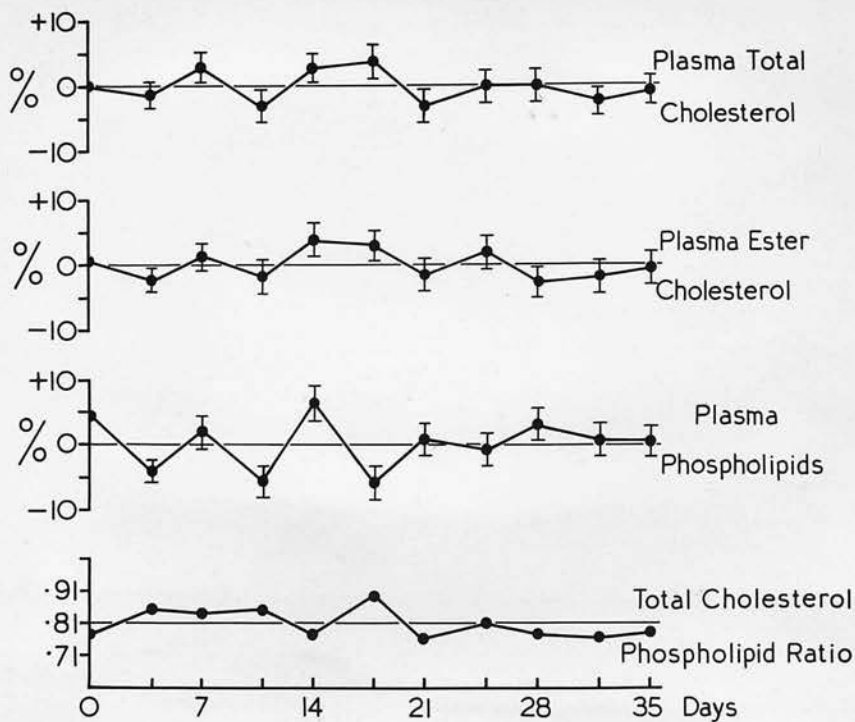
Fig. 11. The mean changes in the α : β lipoprotein
ratio of 12 healthy young women during
the menstrual cycle. The high ratio
at ovulation is due both to an increase
in α-lipoprotein cholesterol and a
decrease in β-lipoprotein cholesterol.
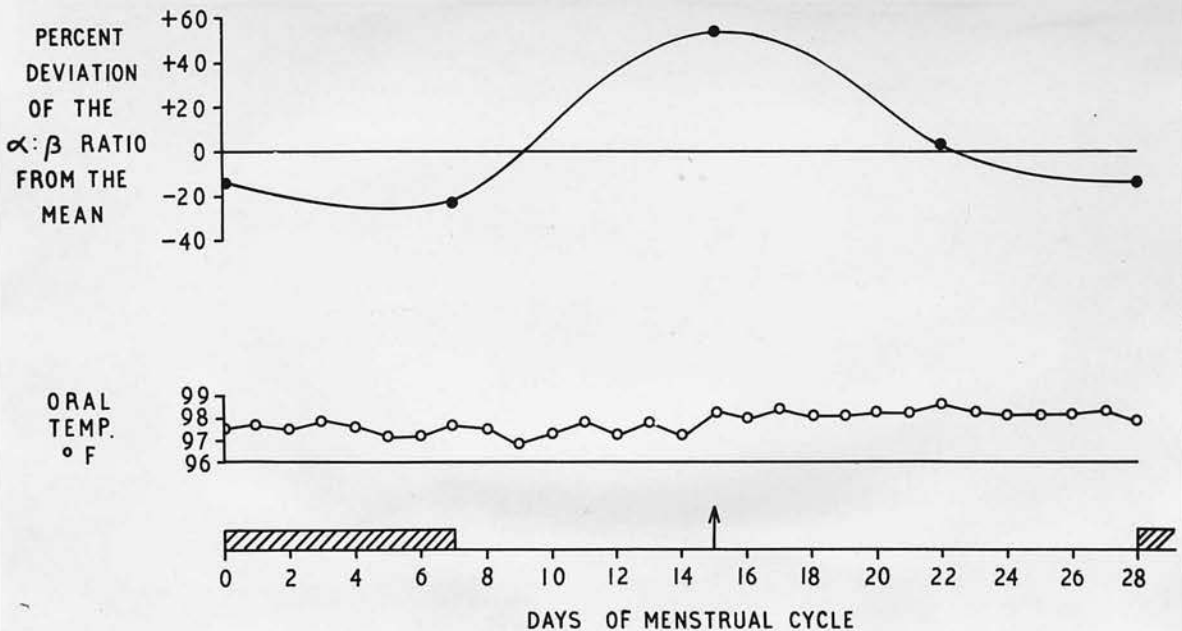
with the digitonin precipitation procedure,
did not consider that there was any consistent
elevation of plasma total cholesterol; and
Boyd (1934), who reviewed these early studies,
considered that the discrepancies were largely
a function of the medium analysed (whole blood,
red cells, serum or plasma) and concluded that the
most important change was a twofold increase in
neutral fat but that there was no significant
elevation of total cholesterol. During the
last 20 years there have been few studies of the
circulating lipids in pregnancy and the puerperium
and the belief that hypercholesterolaemia prevails
in pregnancy has not been firmly established.
The majority of investigators have studied the
last few weeks of pregnancy or have made their
determinations on pooled blood or on different
groups of women at each month.

The subjects studied were 12 normal
primigravidae between the ages of 18 and 31 years,
mean age 24 years. The women, 13 in number, were
selected from the Antenatal Clinic of the Royal
Simpson Memorial Maternity Pavilion; one was
subsequently discarded from the study on account
of abortion at the 12th week of her pregnancy.
The conditions for the selection were that the
women/

women should be primigravid, less than 12 weeks
pregnant, anticipating confinement in hospital
and obstetrically and medically healthy.   All
12 women attended at two-weekly intervals from
the 9th week until confinement.   Blood samples
were taken at the same time of day every 2 weeks
and the patient was instructed to avoid food or
fluid during the preceding 3 hours.   A blood
sample was/taken during labour, within 12 hours
of birth, on the 3rd, 7th and 14th post-partum
days, and 6, 12, and 20 weeks after delivery;
the last four samples were not taken at any
regular time in the day but when the mother found
it most convenient to leave her baby.

At no time can an exact relationship
be assumed between the supposed week of pregnancy
and the actual stage of gestation which has been
reached;   it was therefore considered, when
assembling the results, more accurate to start
calculation of the mean values from a defined
point, such as birth, than from a supposed one,
such as the 9th week.   The mean number of days
between the specimen taken during labour and the
preceding specimen was 8 and thus this latter
sample was regarded as representative of the
39th week, and for each earlier sample 2 weeks
were/

were deducted.   Two of the women were a little uncertain of the date of the first day of their last menstrual period, but it is likely that this variable has been obviated by assuming the gestation period to be 40 weeks and calculating the mean values in retrograde manner as described. The babies weighed between 6 lbs. 1 oz. and 8 lbs. 12 oz. and there were no obvious signs of pre- or post-maturity.

Hypercholesterolaemia developed in all 12 women and was maximal during the third trimester (Fig. 12) (Oliver and Boyd, 1955a). Between the 7th and 8th months the mean plasma total cholesterol was elevated by 50% ($P < 0.01$); ester cholesterol also rose by 50% and free cholesterol by 59%.   The mean plasma total cholesterol fell before labour and nearly reached the initial level by the 4th postpartum month ($P < 0.01$).   The plasma phospholipids and the C/P ratio increased by 25% ($P < 0.01$) by the 7th and 8th months of pregnancy (Fig. 12), and subsequently fell at labour.   The ratio of cholesterol between the α and β lipoprotein fractions also rose significantly ($P < 0.02$) by the 8th month (Fig. 13).   By the 20th post-partum week/

Fig. 12.   The mean changes in the circulating
            cholesterol and the C/P ratio of 12
            healthy young primigravidae during
            pregnancy and the puerperium.
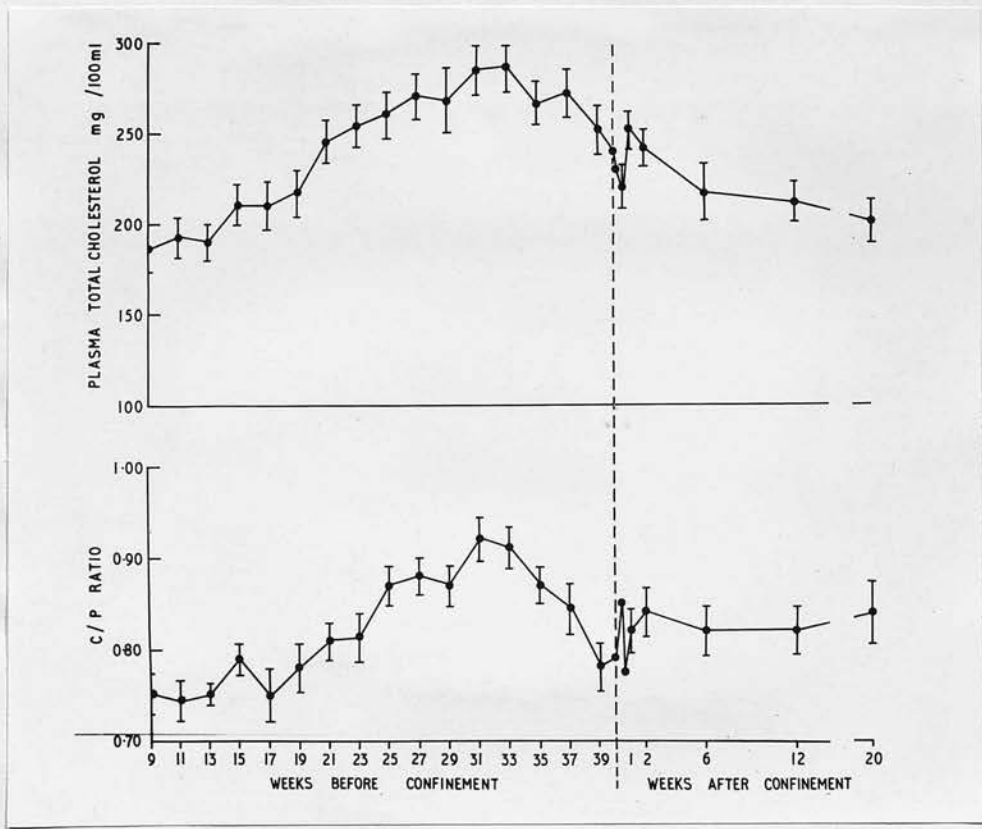
Fig. 13.
The mean changes in the circulating
lipoprotein cholesterol of 12 healthy
young primigravidae during pregnancy
and the puerperium.

(The serum is applied to the filter
paper at the point represented by the
vertical line and is induced to migrate
towards the anode represented by the +).

CHOLESTEROL ATTACHED TO THE β AND α LIPOPROTEINS
BEFORE, DURING , AND AFTER PREGNANCY, AS DETERMINED
BY FILTER PAPER ELECTROPHORESIS.
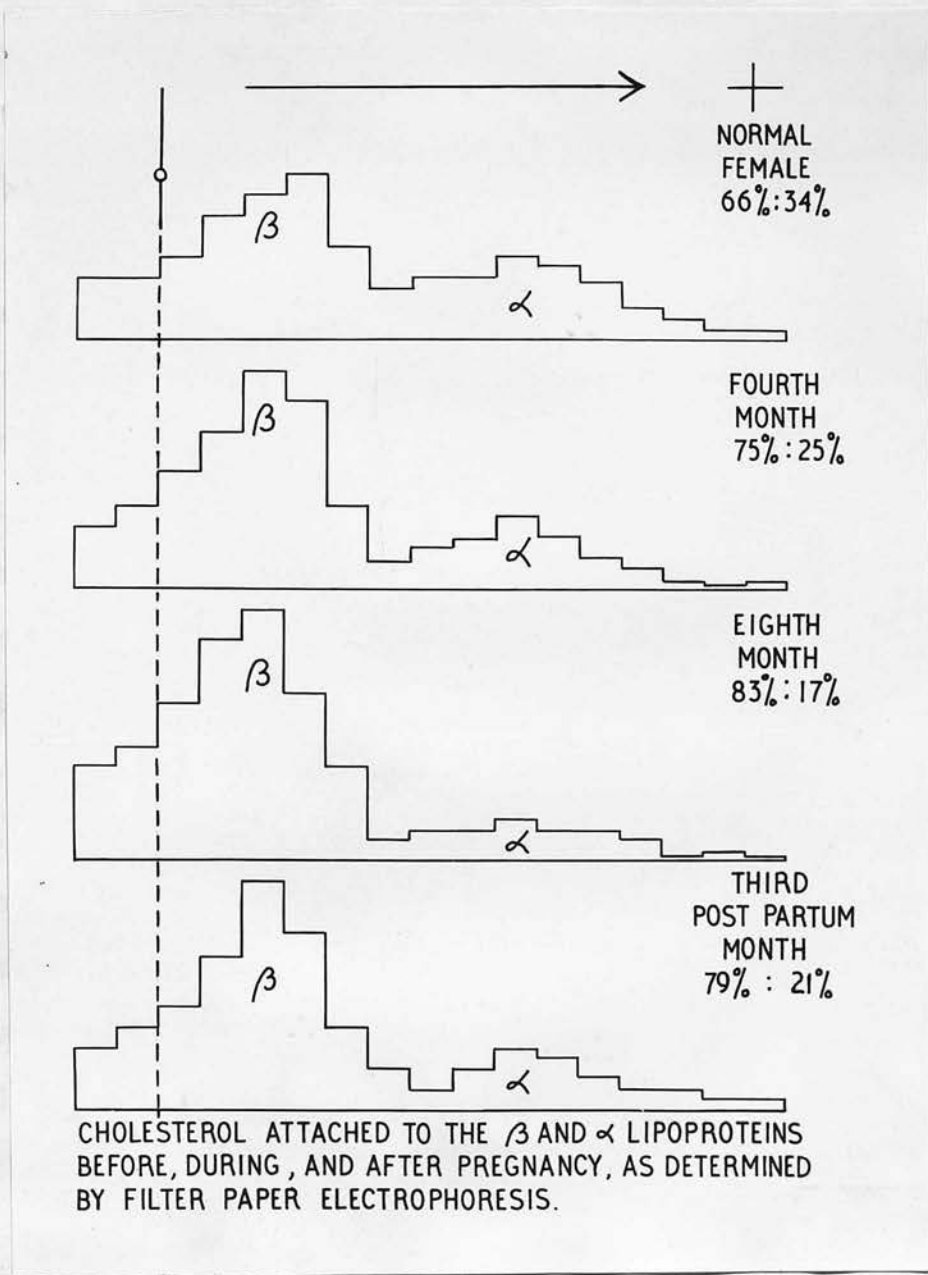
week, when all but one of the women had ceased
lactation and all had had at least one menstrual
period, none of these mean values had quite
returned to the levels observed during the first
trimester.   These slightly higher levels are
not statistically significant for this small group,
and no attempt was made to relate them to any
particular phase of the first post-partum menstrual
cycle.   The mean increase in weight of the 12
women during pregnancy was 36 ± 8 lb. and by the
20th post-partum week it had fallen by 25 ± 6 lb;
thus the mean weight of the women was 9 lb. greater
5 months after the pregnancy than it was before
the pregnancy.   There was no correlation between
the weights during the post-partum period and the
levels of the circulating lipids.   Eight boys
and 4 girls were born to the 12 women and there
was no relationship between the degree of hyper-
cholesterolaemia in the mother and the sex or the
birth weight of the child.

iii.   Post-menopausal years.

The circulating lipids have been studied
in 75 women from the age of 40 (Table VIII);
the subjects were miscellaneous patients who had
no evidence of cardiac, renal, hepatic or metabolic
disease./

Table VIII. The mean plasma cholesterol and C/P ratio in 75 apparently healthy women.

Decades	Numbers	Mean Age	Plasma Cholesterol mg.%	Standard Deviation	Range of Plasma Cholesterol	C/P Ratio	Standard Deviation	Range of C/P Ratio
40 - 49	20	40	184	± 39	103-248	0.79	± 0.09	0.66-0.94
50 - 59	20	54	226	± 44	164-295	0.91	± 0.11	0.68-1.02
60 - 69	24	63	198	± 42	143-292	0.83	± 0.11	0.61-0.99
70 +	11	75	180	± 52	114-300	0.79	± 0.09	0.69-0.92

disease.   No significant difference was observed
between the 40-49, 60-69 and over 70 age groups,
but the plasma cholesterol and C/P ratio of the
50-59 age group was significantly higher than
the other decades (Oliver & Boyd, 1953b).   This
unexpected observation has recently been confirmed
by Adlersberg et al (1956).   Explanation of this
rise in the plasma lipids in the first post-
menopausal decade and of the subsequent fall in
later decades is not readily forthcoming, but might
be related to the hormonal changes associated
with the menopause and the withdrawal of
oestrogens at that time (Page 97).   These in-
fluences of age and sex and the normal cholest-
erol pattern in the human are shown diagram-
matically in Fig. 14 .

6. The influence of race.

It is difficult to dissociate the effect
of primary racial genetic characteristics from
environmental habits, which could be responsible
for most of the racial differences in the cir-
culating lipids and lipoproteins.   The economic
status and standard of living, and the social
and religious habits of various races differ
widely;   these environment factors determine the
quantity/

Fig. 14.   A diagrammatic representation of the
influence of age and sex on the circ-
ulating cholesterol in health.   The
unbroken line represents males and the
broken line represents females;   P
indicates the level of the plasma
cholesterol during the last trimester
of pregnancy.
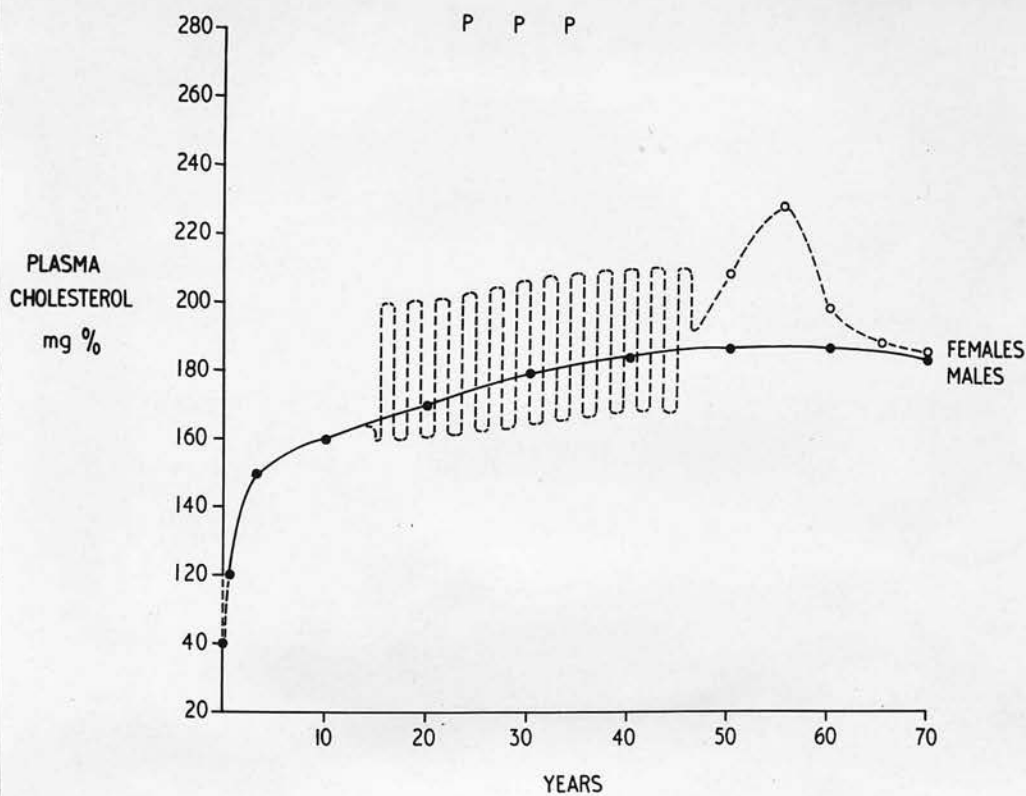
quantity and quality of the diet, and so
influence the circulating cholesterol levels very
considerably.   Elaborate epdemiological studies
have indicated that there exists a direct relation-
ship between an increase in dietary fat and
standard of living with an increase in circulating
cholesterol (Keys and Anderson, 1954;   Keys et al,
1955;   Bronte-Stewart et al, 1955).

7. The influence of diet.

a. Dietary cholesterol.

The circulating cholesterol in men is
largely independent of dietary cholesterol.
Ingestion of excess cholesterol produces only
slight and transient elevation of plasma cholest-
erol (Collen et al, 1949;   Keys, 1949), although
very large quantities of cholesterol will cause a
greater increase in circulating cholesterol
(Messinger et al, 1950).   Reduction of dietary
cholesterol does not produce any significant
decrease in plasma cholesterol (Keys et al, 1950).

b. Dietary fat.

Diets rich in fat, but not necessarily
in cholesterol, are associated with high levels
of circulating cholesterol (Widal et al, 1913;
Keys et al, 1955).   In most civilised countries
more/

more than one-third of the total caloric intake
is derived from fat and the average cholesterol
levels are higher in these countries than in
more primitive communities.   Reduction of total
dietary fat will lower circulating cholesterol
(Mellinkoff et al, 1950) and a rice-fruit diet,
devoid of cholesterol and fat, produces a
substantial fall (Kempner, 1948).

Until recently animal (saturated) and
vegetable (unsaturated) fats were regarded as
interchangeable so far as their effect on the
circulating lipids and lipoproteins was concerned
(Hildreth et al, 1950), but now there is
accumulating evidence to indicate that the
physiological levels may be dependent in part on
the relative, if not on the absolute, intake of
essential or unsaturated fatty acids.   Nutrition-
al studies on vegetarians compared with non-
vegetarians have indicated that high plasma
cholesterol levels are closely associated with
the intake of saturated fatty acids and lack of
unsaturated fatty acids (Hardinge and Stare,
1954).   The addition of unsaturated vegetable
oils to the diet reduces the level of plasma
cholesterol/

cholesterol (Kinsell et al, 1952, 1953) and lack of unsaturated fatty acids appears to result in elevation of the plasma cholesterol (Kinsell et al, 1956). However, complete starvation causes mobilisation of fat reserves, lipaemia, increase in the circulating cholesterol, the C/P ratio (Kartin et al, 1944) and an increase in low density lipoproteins (Rubin and Aladjem, 1954).

B. THE CIRCULATING LIPIDS AND LIPOPROTEINS IN PATIENTS WITH ISCHAEMIC HEART DISEASE.

Evidence has been presented earlier that there are large amounts of cholesterol in athero-sclerotic arterial lesions and that this lipid is largely, if not entirely, derived from the plasma. Conditions where there is elevation of the cir-culating cholesterol are associated with a high incidence of atherosclerosis and ischaemic heart disease (see Page 41). It would seem desirable, therefore, to determine whether there is any abnormality in the circulating lipids and lipo-proteins in patients who have developed the clinical manifestations of coronary atherosclerosis

1. Plasma cholesterol and C/P ratio.

Three studies in the United States have suggested that there is elevation of the plasma cholesterol and probably of the C/P ratio in patients/

patients with ischaemic heart disease (Morrison
et al, 1948; Gertler et al, 1950; Steiner et al,
1952). A larger study (Oliver and Boyd, 1953b)
precisely controlled in respect of age and sex,
was undertaken in order to determine the relation-
ship between cholesterol and ischaemic heart
disease in Britain where the dietary habits differ
slightly from those in the United States.

The subjects were 200 consecutive
admissions with ischaemic heart disease and 200
miscellaneous controls. In the coronary disease
group, there was electrocardiographic confirmation
of myocardial infarction in 170, and of ischaemia
before or after the Master two-step test in 30 who
presented clinically with angina of effort;
any subject who lacked electrocardiographic
confirmation of ischaemic heart disease was ex-
cluded. Adequate controls were very difficult to
obtain from a hospital population, but were care-
fully selected members of the medical and scien-
tific staff, and from convalescent inpatients,
who had no history or clinical features of cardiac,
renal, hepatic or metabolic diseases. The
coronary disease group was completed first, and
the mean age of each decade of both sexes was
determined;/

determined; the control group was then completed
so that the mean age and number of cases in each
decade corresponded with the coronary disease
group. Blood samples taken during anticoagulant
therapy or within five weeks of the occurrence
of myocardial infarction have been excluded from
the study. All subjects received a light ward
or weight-reducing diet.

The levels of the plasma lipids of each
group have been contrasted by decades and ex-
pressed separately for the sexes; the mean levels
and standard errors are shown in Figs. 15 - 18.
Fig. 15 shows the mean plasma cholesterol and
Fig. 16 the mean plasma C/P ratio in the men,
and Table IXa shows the actual values of these
means together with the standard deviations and
ranges. There was significant elevation of the
plasma cholesterol and the C/P ratio in men with
coronary disease in all decades and the maximum
elevation occurred between the ages of 30 and 39.
Fig. 17 represents the mean plasma cholesterol
and Fig. 18 the mean C/P ratio in the women,
and Table IXb shows the actual values of these
means together with the standard deviations and
ranges. As for men, there was significant
overall/

Fig. 15. The mean plasma cholesterol levels in
          149 consecutive male admissions for
          ischaemic heart disease and in a
          matched control group;   the number
          of cases in each group for each decade
          is also shown.

MALES

PLASMA
TOTAL CHOLESTEROL
IN mg.%

DECADES	30 -39	40 -49	50 -59	60 -69	70+
NUMBER OF CASES	12	36	52	37	12

CORONARY ARTERY DISEASE GROUP

CONTROL GROUP

The mean plasma C/P ratio in 149
consecutive male admissions for ischaemic
heart disease and in a matched control
group;   the number of cases in each
group for each decade is also shown.

MALES

PLASMA
TOTAL-CHOLESTEROL:
PHOSPHOLIPID RATIO
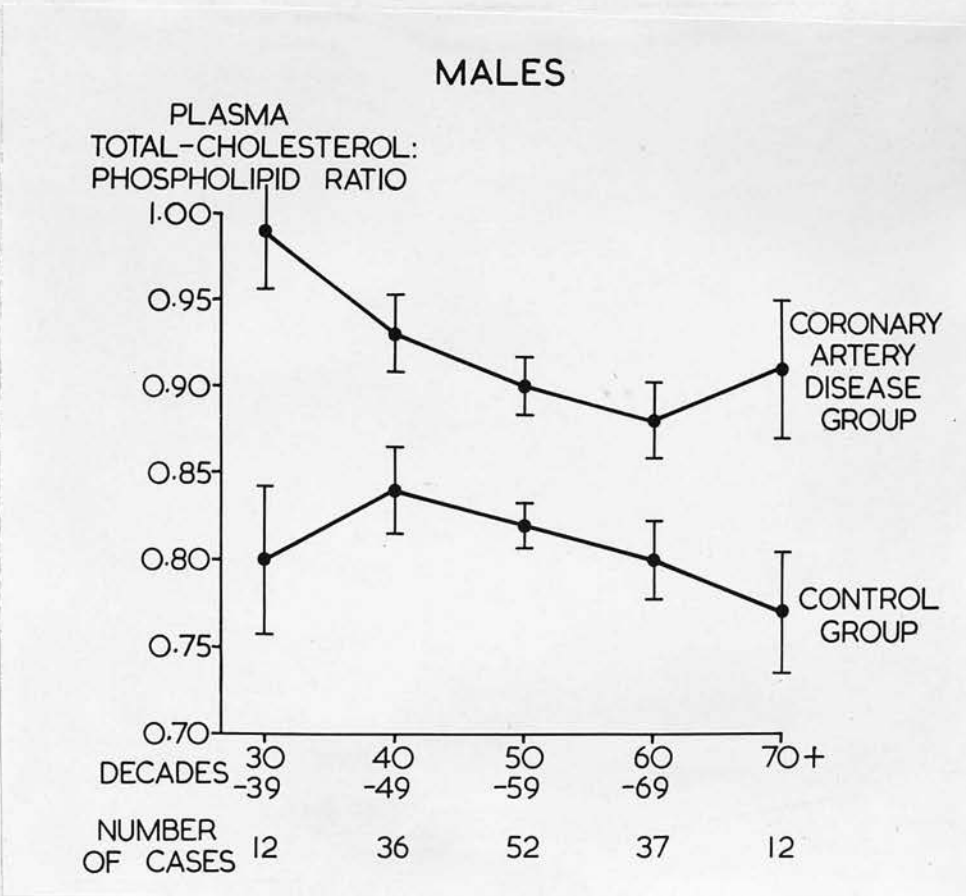
Table IXa.   The circulating lipids in 149 consecutive men with ischaemic heart disease and 149 controls.

CORONARY DISEASE GROUP

Decades	Numbers	Mean age	Plasma Cholesterol mg%	Standard Deviation	Range of Plasma Cholesterol	C/P Ratio	Standard Deviation	Range in C/P Ratio
30 - 39	12	36	278	± 64	201 - 428	0.99	± 0.12	0.79 - 1.23
40 - 49	36	45	243	± 60	132 - 405	0.93	± 0.14	0.58 - 1.16
50 - 59	52	54	224	± 59	120 - 424	0.90	± 0.12	0.63 - 1.17
60 - 69	37	64	223	± 57	117 - 430	0.88	± 0.14	0.53 - 1.17
70 +	12	75	214	± 66	134 - 340	0.91	± 0.14	0.66 - 1.22

CONTROL GROUP

Decades	Numbers	Mean age	Plasma Cholesterol mg%	Standard Deviation	Range of Plasma Cholesterol	C/P Ratio	Standard Deviation	Range in C/P Ratio
30 - 39	12	36	174	± 22	127 - 206	0.80	± 0.15	0.49 - 0.98
40 - 49	36	45	180	± 33	110 - 234	0.84	± 0.15	0.52 - 1.16
50 - 59	52	54	168	± 30	106 - 230	0.82	± 0.10	0.53 - 1.07
60 - 69	37	64	166	± 42	100 - 265	0.80	± 0.14	0.51 - 1.30
70 +	12	75	171	± 34	124 - 242	0.77	± 0.12	0.54 - 0.90

Fig. 17. The mean plasma cholesterol levels in
51 consecutive female admissions for
ischaemic heart disease and in a matched
control group; the number of cases in
each group for each decade is also shown.
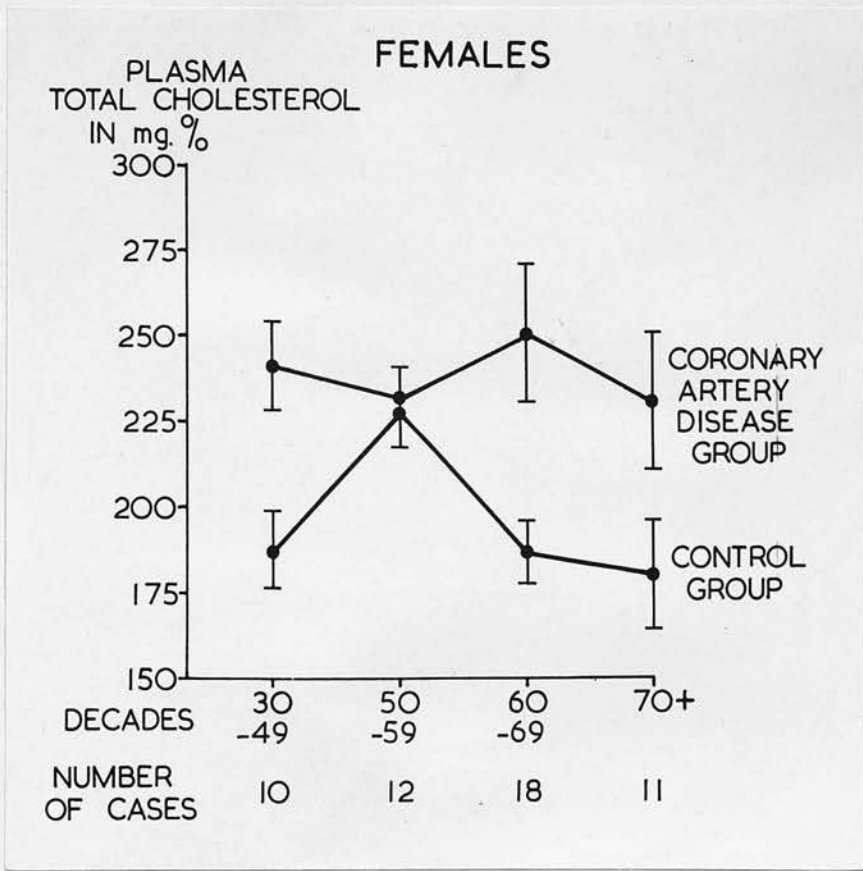
Fig. 18. The mean plasma C/P ratio in 149
consecutive female admissions for
ischaemic heart disease and in a matched
control group;   the number of cases in
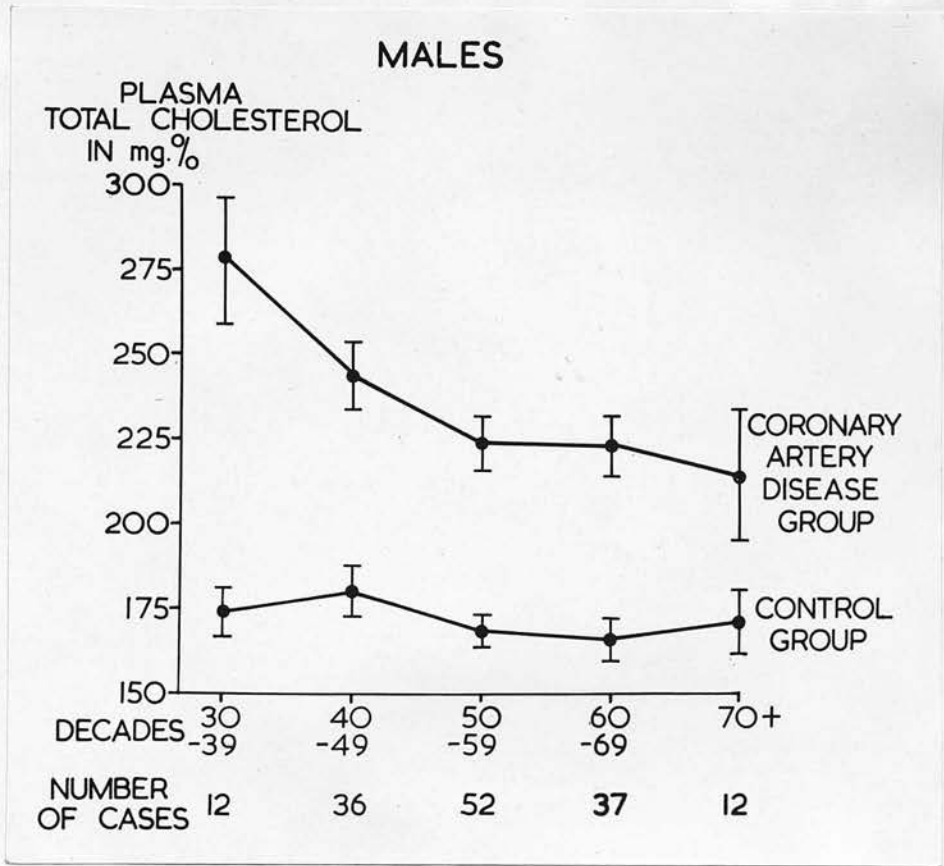each group for each decade is also shown.

FEMALES
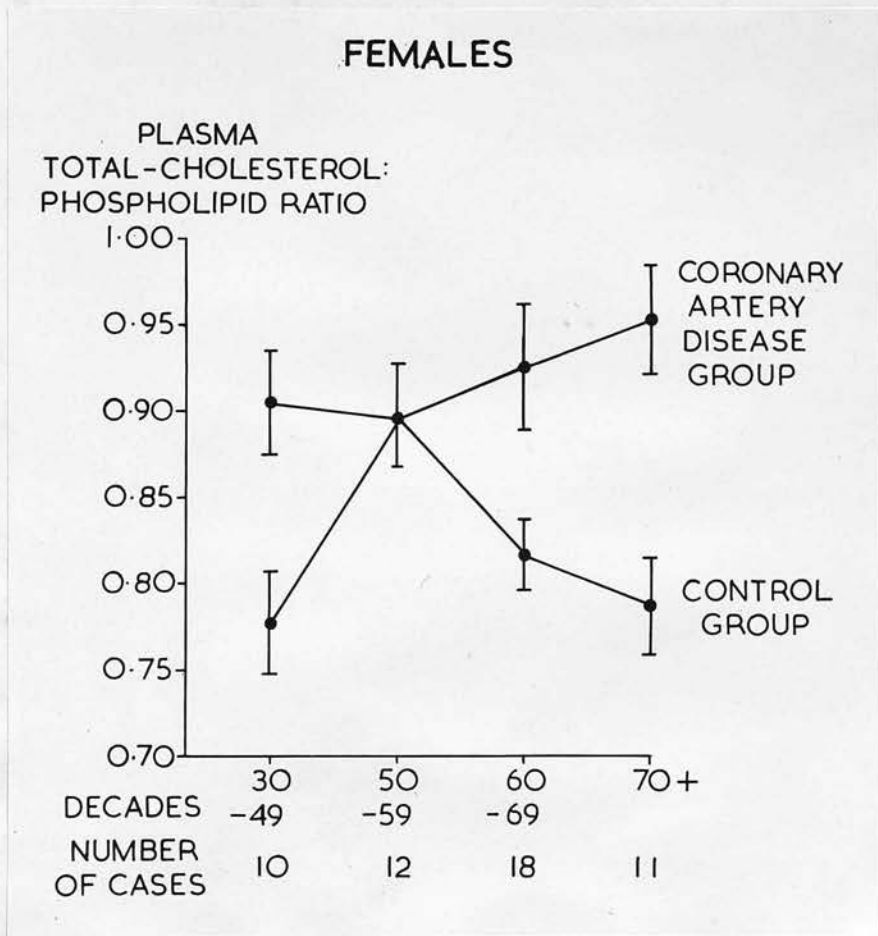
Table IXb. The circulating lipids in 51 consecutive women with ischaemic heart disease and 51 controls.

Decades	Numbers	Mean age	Plasma Cholesterol mg%	Standard Deviation	Range of Plasma Cholesterol	C/P Ratio	Standard Deviation	Range in C/P Ratio
CORONARY DISEASE GROUP								
30 - 49	10	42	241	± 39	206 - 342	0.91	± 0.11	0.75 - 1.09
50 - 59	12	54	232	± 33	160 - 282	0.90	± 0.09	0.80 - 1.14
60 - 69	18	64	251	± 88	167 - 432	0.93	± 0.16	0.59 - 1.22
70 +	11	75	239	± 65	188 - 360	0.96	± 0.10	0.75 - 1.23
CONTROLS GROUP								
30 - 49	10	42	187	± 36	135 - 248	0.78	± 0.09	0.66 - 0.94
50 - 59	12	54	228	± 40	164 - 295	0.90	± 0.10	0.68 - 1.02
60 - 69	18	64	187	± 40	143 - 270	0.82	± 0.09	0.66 - 0.99
70 +	11	75	180	± 52	114 - 300	0.79	± 0.09	0.69 - 0.92

overall elevation of these values in women with
coronary disease in all decades except the sixth
when the values of the control group and coronary
disease group were identical (see Page 65).
In women, there was no hypercholesterolaemia in
the earliest decade of the study.

Whereas there was significant elevation
of plasma cholesterol and the C/P ratio in the
coronary disease group, there was also considerable
overlap in the levels of the two groups.   Thus,
in 19% of the apparently healthy men either the
plasma cholesterol was above 205 mg% or the C/P
ratio was greater than 0.94 (these figures are the
mean values + one standard deviation), and in
4% either the plasma cholesterol was above 239 mg%
or the C/P ratio was greather than 1.07 (these
figures are the mean values + two standard
deviations).   Similarly, 29% of the men with
coronary disease had either plasma cholesterol
levels lower than 205 mg% or C/P ratio levels
less than 0.94.   However, only 15% of the 48 men
with ischaemic heart disease under the age of 50
demonstrated either a low plasma cholesterol or
a low C/P ratio.   The overlap between the control
and coronary groups is further reduced when
lipoprotein analysis are included, and under the
age/

age of 50 only between 5 and 9% of patients with
ischaemic heart disease had entirely normal
levels (see Page 79).   The most significant eleva-
tion in the plasma cholesterol and C/P ratio
occurred in men who had developed ischaemic
heart disease under the age of 40 (see Page 77). A
marked degree of hypercholesterolaemia may favour
the premature development of ischaemic heart
disease, and exposure to a high plasma cholesterol
for a short period may have the same deleterious
effect on the coronary arteries that might result
from milder and more long-standing elevation.

  2.   Serum lipoproteins.
     a.   In the human.

The circulating lipids do not exist free
in plasma but are attached to protein;   these
lipids are almost entirely bound to the α and β
globulins and these lipid-protein macromolecules
are termed the α and β lipoproteins (see Page 48).
It would seem desirable to determine the dis-
tribution of cholesterol between these two lipid
carriers in patients with heart disease and other
conditions associated with hypercholesterolaemia.

The subjects (who were different from those
reported on the preceding pages) were 50 consecutive
men/

men with ischaemic heart disease under the age
of 50, 50 miscellaneous controls, 4 men and 4
women with xanthoma tuberosum, 3 men and 1 woman
with xanthoma tendinosum, and 2 men with
"essential hyperlipaemia".   The control group,
which was matched by age to the coronary group,
consisted of members of the medical and scien-
tific staff and convalescent inpatients, who had
no history of clinical features of cardiac, renal,
hepatic or metabolic disease.   In the coronary
disease group, there was electrocardiographic
confirmation of myocardial infarction in 39 and
of ischaemia before or after the Master two-step
test in 11 who presented clinically with angina
of effort;   these men were not receiving any
therapy at the time of the estimation and none
had had a myocardial infarct within three months
of the determinations, which were repeated on all
the subjects on several occasions.

        The lipoprotein pattern in the coronary
disease group and xanthoma group is contrasted
with the normal pattern in Fig. 19.   The results
expressed in Table X indicate that the concen-
tration of cholesterol attached to the β-lipo-
protein fraction is increased in the coronary
disease/

Fig. 19.   The circulating lipoprotein cholesterol
          in healthy men, in men with ischaemic
          heart disease and in 12 patients with
          xanthomatosis.

          (The serum is applied to the filter
          paper at the point represented by the
          vertical line and is induced to migrate
          towards the anode represented by the +).

CHOLESTEROL ATTACHED TO THE β AND α LIPOPROTEINS AS
DETERMINED BY FILTER PAPER ELECTROPHORESIS
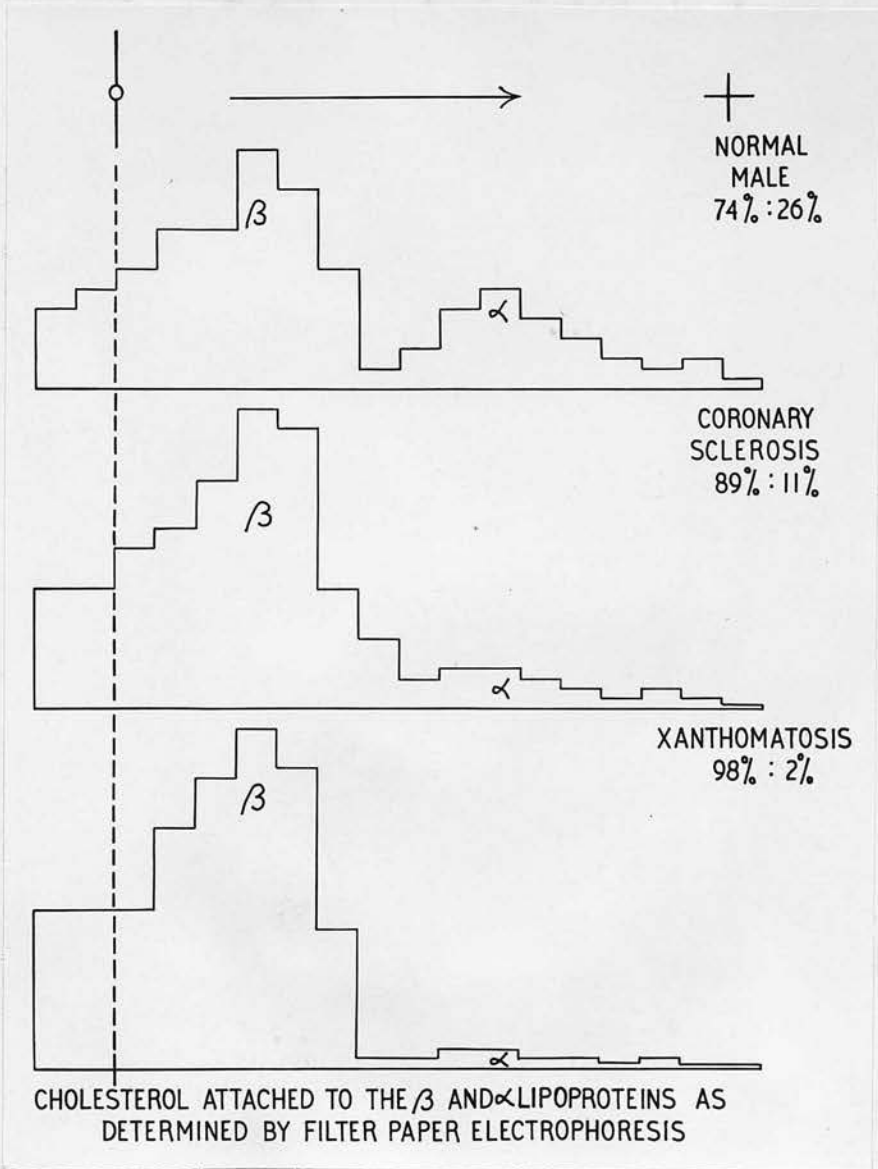
Table X. The percentage distribution of cholesterol between the α and β lipoproteins, expressed as the $\alpha:\beta$ lipoprotein ratio, in 50 consecutive patients with ischaemic heart disease under the age of 50, in a matched control group and in miscellaneous hypercholesterolaemic conditions.

	Number of cases	Mean age	Mean $\alpha:\beta$ lipoprotein ratio	Standard Deviation	Mean Plasma Cholesterol mg%	Mean C/P Ratio
Normal men	50	41	28:72	±9.8	193	0.82
Men with coronary sclerosis	50	41	9:91	±6.1	270	1.04
Xanthoma tuberosum	8	40	7:93	-	394	1.01
Xanthoma tendinosum	4	35	3:97	-	442	1.20
"Idiopathic hyperlipaemia"	2	37	1:99	-	330	1.13

disease group when contrasted with the control group, and the concentration of cholesterol attached to the α-lipoprotein fraction is correspondingly decreased (Oliver and Boyd, 1955b); this study has been extended to a larger, but uncontrolled, group of patients with ischaemic heart disease under 50 (see Page 77 and Table XII). The men with coronary disease were all comparatively young (mean age 41) and thus the majority exhibited hypercholesterolaemia (see Pages 70 & 77). It is probable that the rather greater increase in the β-lipoprotein cholesterol in the present investigation when compared with studies of older men with ischaemic heart disease (Nikkilä, 1953; Rosenberg et al, 1954) is largely due to this hypercholesterolaemia, and that, whereas the group can fairly be regarded as representative of the young coronary population, it is probably not a cross section of the older coronary population in which marked hypercholesterolaemia is rather less frequent.

b.   Species differences in relation to atherosclerosis.

There are marked and interesting differences between the species in the distribution of cholesterol between the serum lipoproteins/

lipoproteins in relation to the development of atherosclerosis (Table XI)(Boyd and Oliver, 1956); and, although not directly related to the investigation of patients with ischaemic heart disease, this comparative study indicates a positive correlation between an increasing concentration of β-lipoprotein cholesterol and the tendency to develop atherosclerosis. In the rat it is difficult to increase the plasma cholesterol by feeding cholesterol or fat, and until recently this species was regarded as immune from atheroma; however, it has now been shown that exposure to cold (1 - 3°C) for 12 months results in hypercholesterolaemia and the development of typical atherosclerotic lesions in the intima of the aorta (Sellers and You, 1956). In the rat the percentage distribution of cholesterol between the α and β lipoproteins is approximately 90:10. By contrast the corresponding percentage distribution in normal dog plasma is 70:30, and typical atherosclerotic lesions can readily be induced by cholesterol feeding provided thyroid activity is depressed at the same time (Steiner & Kendall, 1946). In the normal rabbit the percentage distribution of cholesterol between the/

Table XI. Increasing concentrations of cholesterol on the β-lipoprotein ratio, or a low $\alpha:\beta$ lipoprotein ratio, appear to be related to the development of atherosclerotic lesions in different species.

	Cholesterol mg% (average figure)	Average $\alpha:\beta$ lipoprotein ratio	Atherosclerotic lesions
Human: Coronary disease In health	240 + 180	10 : 90 25 : 75	Spontaneous lesions
Fowl	70	40 : 60	Spontaneous lesions
Rabbit	40	50 : 50	With cholesterol feeding only
Dog	140	70 : 30	With cholesterol feeding + thyroid depression
Rat	50	90 : 10	Rarely produced: Exposure to cold for 1 year.

the α and β lipoproteins is 50:50, and athero-
sclerotic lesions can easily be induced by
moderate cholesterol feeding.   Birds most closely
resemble the human in that atherosclerosis may
develop spontaneously (Dauber, 1944) and the
$\alpha:\beta$ lipoprotein ratio is approximately 40:60.
In the human, virtually every adult shows some
degree of atheroma in the aorta and there is
therefore no strict normal group for study;
comparisons of patients and "normals" are actually
comparisons between different degrees of severity
of atherosclerotic lesions.   However, the $\alpha:\beta$
lipoprotein ratio in a group of healthy men was
28:72 (see Table X) in contrast to 10:90 in men
with established coronary disease (Oliver & Boyd,
1955b).

3.   The circulating lipids and lipoproteins
      in young patients with ischaemic heart
      disease and their significance.

The presence of marked abnormality of
the circulating lipids and lipoproteins in young
patients with ischaemic heart disease is further
emphasised by the study of 237 such patients, who
were referred to the Department of Cardiology in
the Edinburgh Royal Infirmary during the last 5
years.   These patients all had electrocardio-
graphic/

graphic evidence of myocardial infarction or
ischaemia, and the results of their lipid and
lipoprotein analysis are shown in Table XII.

Table XII.　　The mean circulating lipids and
lipoproteins in patients with
ischaemic heart disease under the
age of 50.

	MALES		FEMALES	
	40	40-49	40	40-49
Number of cases	67	125	20	25
Plasma cholest-erol (mgm%)	275	255	299	271
Standard Deviation	±68	±41	±114	±94
C/P ratio	1.03	1.00	1.15	0.96
Standard Deviation	±0.03	±0.04	±0.07	±0.06
Number of cases	32	36	13	15
$\alpha : \beta$ lipo-protein ratio	8:92*	8:92†	11:89*	14:86†

* The standard deviation of the β-lipoprotein
cholesterol, when expressed in mg%, represents
a change of ± 5% in the $\alpha : \beta$ lipoprotein ratio.

† The standard deviation of the β-lipoprotein
cholesterol, when expressed in mg%, represents
a change of ± 8% in the $\alpha : \beta$ lipoprotein ratio.

In 140 healthy subjects of both sexes
under the age of 50 the mean plasma cholesterol
was/

was 182 ± 38 mg% and the mean C/P ratio was
0.81 ± 0.13, and in 76 similar subjects the mean
$\alpha : \beta$ lipoprotein ratio was 22:78 $\pm$ 7.   These
subjects were carefully selected members of the
medical and scientific staff, and convalescent
inpatients who had no history or clinical features
of cardiac, renal, hepatic or metabolic diseases;
their lipid and lipoprotein analyses have been
used as controls in some of the studies already
mentioned.   The addition of one standard deviation
to these means increases the plasma cholesterol
level to 220 mg%, the C/P ratio to 0.94 and the
$\alpha : \beta$ lipoprotein ratio to 15:85.   Regarding
these levels as the upper limits of "normal",
the percentage of apparently healthy young adults
and the percentage of young patients with isch-
aemic heart disease with "normal" circulating
lipids and lipoproteins have been assessed and the
results are shown in Table XIII.

Table XIII./

Table XIII. The percentage of apparently healthy young adults and the percentage of young patients with ischaemic heart disease with "normal" circulating lipids and lipoproteins.

	Under 40 years		40-49 years	
	Control Group	Coronary Group	Control Group	Coronary Group
Number of cases	80	87	60	150
Plasma cholesterol below 221 mg%	91%	11%	87%	26%
C/P ratio below 0.95	87%	30%	83%	38%
Plasma cholesterol below 221 mg% + C/P ratio below 0.95	93%	9%	91%	20%
Number of cases	44	45	32	51
$\alpha:\beta$ lipoprotein ratio below 16:84	94%	20%	88%	19%
Plasma cholesterol below 221 mg% + C/P ratio below 0.95 + $\alpha:\beta$ lipoprotein ratio below 16:84	96%	5%	90%	9%

Any/

Any attempt to divide the control group from the coronary disease group on the basis of their circulating lipids and lipoproteins depends on the arbitary choice of certain values to represent the upper limits of normality. Several possibilities were investigated but the division based on the addition of one standard deviation to the mean values of the control group resulted in the most significant separation of the groups. This separation is quite impressive under the age of 40 when only about 5% of each group do not conform to the majority; the division of the groups is not so complete between the ages of 40 and 49 and becomes even less with advancing age – for instance, between 60 and 69 years 44% of the coronary group were found to have normal levels of both the plasma cholesterol and the C/P ratio, although only 10% of the control group had abnormal levels.

When preventitive treatment for ischaemic heart disease becomes available, it will be necessary to develop methods for screening the apparently healthy population in order to discover those who will later develop the disease. It is likely/

likely that analyses of the circulating lipids
and lipoproteins will contribute greatly to such
an investigation of the healthy population.   It
is suggested, moreover, that these analyses are
useful and reasonably reliable adjuvants to the
diagnosis of coronary disease in young patients.

4. Heparin clearing reaction.

        Post-prandial plasma turbidity or lipaemia
normally disappears following the intravenous
administration of heparin (Hahn, 1943);   this
action of heparin can only be initiated in vivo.
Block et al (1951) reported that a small intra-
venous injection of heparin produced less clearing
of lipaemic plasma in 27 males with myocardial
infarction or with atherosclerotic obliteration
of the lower limb vessels when compared with 23
normal men of a slightly younger age group.
They suggested that there may be a relationship
between atherosclerosis and the lack of dis-
appearance of alimentary lipaemia following heparin.
It was thought that this observation merited
further investigation.

        One hundred subjects were studied.   With
the exception of a few ill patients, 50 were
consecutive admissions with electrocardiographic
confirmation/

confirmation of myocardial infarction or
ischaemia, and 50 were miscellaneous hospital
inpatients without evidence of cardiac, hepatic,
renal or metabolic diseases.   The subjects
received for breakfast 100 g. of fat in the form
of 40% cream and developed a consistent post-
prandial lipaemia.   The turbidity of the plasma
was measured by determining its optical density
(on a Unicam Absorptiometer) before, 3 hours after
and $3\frac{1}{4}$ hours after the fat meal.   A small intra-
venous injection (300 I. U.) of heparin was given
at the height of the postprandial lipaemia 3 hours
after the fat meal.

Heparin induced less clearing of the
lipaemia in the coronary disease group than in
the control group (Table XIV) (Oliver and Boyd,
1953c).   In Fig. 20 the average optical density
of each group has been diagrammatically superimposed
on the normal absorption curve following the inges-
tion of 100 g. of fat.   Thus, at $3\frac{1}{4}$ hours the
change in the translucency of lipaemic plasma res-
ulting from the heparin injection can be contrasted
 with the level of lipaemia before heparin was ad-
ministered.   The course of normal lipaemia was
studied in 20 subjects, and the maximum lipaemia
developed/

Table XIV.   The mean percentage clearing of lipaemic plasma
15 minutes after heparin.

M E N

	Number of cases	Mean age	Percentage clearing	Standard Deviation	Range in percentage clearing
Coronary group	36	51	30	±24	0 - 84
Control group	36	51	68	±17	30 - 100

W O M E N

	Number of cases	Mean age	Percentage clearing	Standard Deviation	Range in percentage clearing
Coronary group	14	54	35	±21	0 - 76
Control group	14	54	56	±34	0 - 100

Probability that the difference in mean percentage clearing is due to
change :-

Males $P < 0.01$
Females $P < 0.05$

Percentage clearing = $\dfrac{100 \times \text{Preheparin optical density} - \text{Postheparin optical density}}{\text{Preheparin optical density}}$

The optical density of the fasting plasma is subtracted from the pre- and post-
heparin optical densities before the calculation is made.

Fig. 20.   A diagrammatic representation of the
           clearing of alimentary lipaemia induced
           by the intravenous injection of 300 I. U.
           of heparin in patients with ischaemic
           heart disease and in a comparable control
           group.
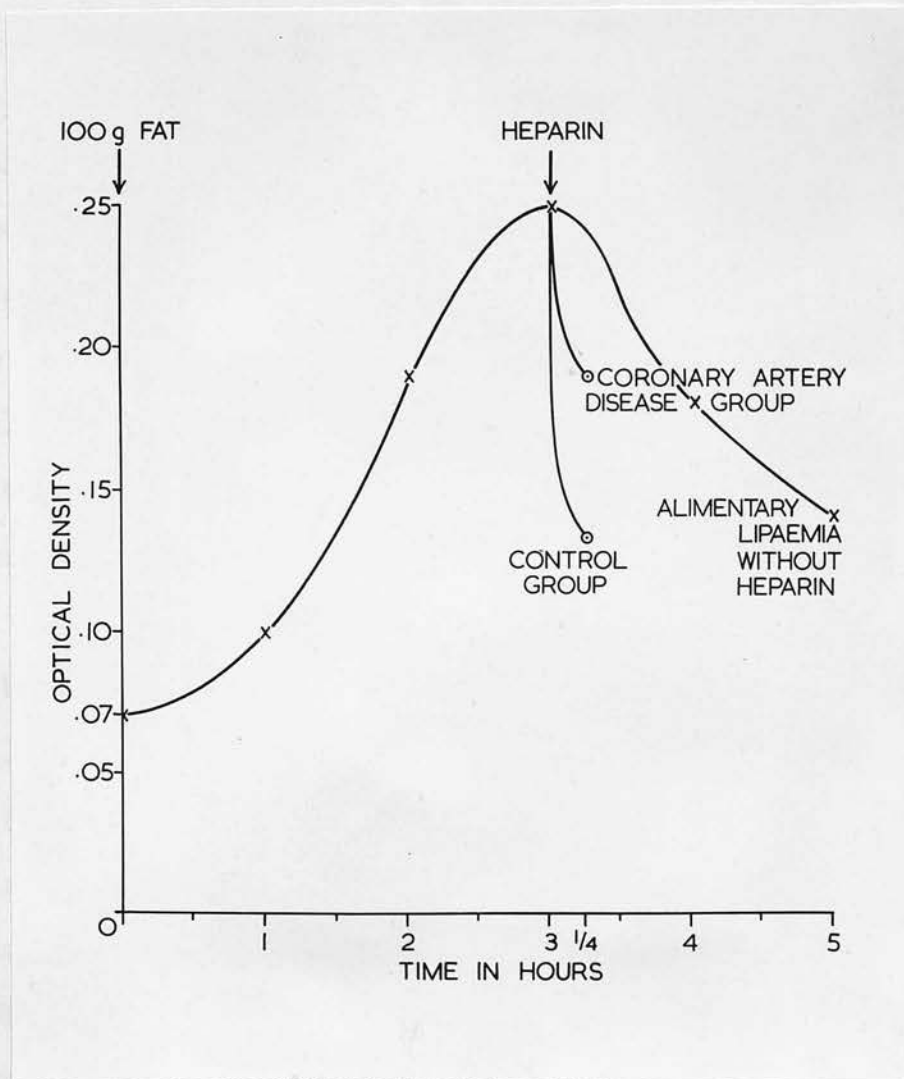
developed usually about 3 hours after the fat
meal and disappeared 3 hours later. There is,
therefore, no reason to believe that there might
be any sudden spontaneous change in the degree
of lipaemia between 3 and $3\frac{1}{4}$ hours after a fat
meal. There was slight overlap of individual
values for percentage clearing from the coronary
disease group into the range of the control group,
and conversely; thus the application of heparin
clearing of lipaemic plasma as an adjuvant to
the diagnosis of coronary artery disease is limit-
ed, perhaps by the difficulty of excluding athero-
sclerosis in the control group. Additional
studies concerning the nature and speed of the
clearing action of heparin have been described
(Oliver & Boyd, 1953c) but do not come directly
into the theme of this thesis.

Explanation of the difference between
the coronary disease and control groups in the
clearing of alimentary lipaemia by a small in-
jection of heparin is not readily forthcoming.
Exogenous heparin apparently requires some tissue
component in addition to plasma in order to
produce a clearing factor. Although heparin has
not been detected in normal plasma, there is con-
siderable/

siderable evidence that a heparin-like sulphated
mucopolysaccharide is present in mast cells, and
the physiological dissolution of chylomicra may
depend upon the presence of an endogenous heparin-
induced clearing factor.   The postprandial lipae-
mia in old age and in subjects with atherosclero-
sis is of longer duration than in the normal, and
this suggests that less endogenous clearing factor
may be available in the presence of atherosclero-
sis.   In this connection there is some histo-
logical evidence to suggest that extracellular
cholesterol depositions occur at areas containing
heparin-like sulphuric acid esters (Faber, 1949),
and thus atherosclerotic changes might restrict
the availability of an endogenous heparin-like
substance and thereby reduce the concentration
of circulating clearing factor.

C.   CONCLUSION.

There is no doubt that the plasma
cholesterol, the C/P ratio and the concentration
of cholesterol on the β-lipoprotein fraction are
all elevated in the majority of patients who have
developed the clinical features of coronary athero-
sclerosis.   These abnormalities are most marked
in patients who develop symptoms under the age of
50/

50 and particularly under the age of 40.   A small
percentage of patients do not exhibit any abnor-
mality in the analysis of the circulating lipids
and lipoproteins and therefore such analyses cannot
be used as diagnostic tests for the presence of
impending ischaemic heart disease; however, they
have proved to be a useful adjuvant towards the
solution of an equivocal clinical diagnosis
particularly in young subjects.   In patients with
ischaemic heart disease, in contrast to healthy
men and women, a more prolonged lipaemia develops
after a meal rich in fat, and the clearing of
this lipaemia by heparin is less efficient and
quick.   Whereas this difference is obvious and
significant, it is not entirely consistent and
also cannot be used as a diagnostic test for the
presence of impending ischaemic heart disease.

These results leave little doubt that
ischaemic heart disease is frequently associated
with some disturbance of the circulating lipids
and lipoproteins. Moreover, a recent elaborate
4 year cooperative survey has shown conclusively
that the circulating lipids and lipoproteins are
abnormal before the development of any of the
clinical features of coronary atherosclerosis.
(Report/

(Report of a cooperative study of the lipoproteins and atherosclerosis, 1956). This association of abnormal circulating lipids and lipoproteins with coronary atherosclerosis, together with the patchy distribution of atherosclerotic lesions, the high incidence of ischaemic heart disease in early middle age, its prevalence in males and the influence of heredity and body build, all indicate that coronary atherosclerosis is not solely an inevitable result of advancing years. It would seem reasonable to postulate that coronary atherosclerosis is an active process and is dependent, at least to some extent, on a disturbance of lipid metabolism.

Four possible explanations for this association of coronary atherosclerosis with a disturbance of lipid metabolism will now be examined:

1.   It might be a chance association. This possibility can be dismissed with considerable confidence in view of the unanimity of several different investigators that such an association exists, and the statistical significance of their results.

2.   It might result from a primary change within the wall of the coronary arteries, or from

a/

a primary disturbance of the physiological
mechanisms which normally prevent the deposition
of fibrin or formation of thrombi.

Resolution of the inflammatory process,
formation of fibrous and hyaline material and
degenerative changes in tissues are often
associated with accumulation of cholesterol esters.
Whereas such changes may account for some of the
cholesterol in the wall of coronary arteries, the
quantity of lipid that accumulates in athero-
sclerotic lesions is so large as to preclude the
possibility that it all originates from the simple
breakdown of intimal tissue (Duff & McMillan, 1951).
Furthermore, it is doubtful whether cholesterol
is synthesised in any quantity, or even at all,
in the arterial wall (Gould, 1951). It is
likely, if any primary change takes place in the
arterial wall, that the site of this change will
act as a nidus for cholesterol deposition rather
than a stimulus to local synthesis of cholesterol.

It is difficult to visualise how a primary
disturbance of the physiological coagulation-
fibrinolysis balance could result in hypercholest-
erolaemia, unless a change in the concentration
of the various accelerators, decelerators and
cofactors,/

cofactors, which maintain the blood in its fluid
state, alters hepatic cholesterol biosynthesis
at the same time.   It seems improbable that
deposition of fibrin or formation of a thrombus
in an artery could upset the biosynthesis of
cholesterol in the liver, unless fibrin is ini-
tially deposited in the hepatic rather than the
coronary arteries and aorta, but there is no
evidence that this is so.

3.   It might contribute directly towards the
development of coronary atherosclerosis. This
has been and is still widely believed.   Hyper-
cholesterolaemia of some degree is nearly always
essential for the production of atherosclerotic
lesions in animals, and the extent and duration
of the hypercholesterolaemia determines the
number and the severity of atherosclerotic lesions.
Moreover, there is a great deal of evidence, review-
ed by Hueper (1944) and brought up to date in the
section on arterial lesions (see Page 12), that
plasma cholesterol passes physiologically through
the vessel wall from the lumen and contributes
to, or may be entirely responsible for, the
cholesterol in the atherosclerotic plaque.   The
patchy/

patchy distribution of atherosclerotic lesions
in the coronary vascular tree indicates that some
parts are more prone to cholesterol deposition
than others and, since the composition of the
plasma which bathes the vascular lining is
essentially the same in all areas, this discrete
distribution of atherosclerotic lesions probably
depends on local differences in the arterial wall
or on local haemodynamic effects.   The nature and
relative importance of various local factors have
not been thoroughly investigated, but variations
in intimal permeability and in the metabolic
activities of the intimal cellular elements in
handling lipids presented to them may be important
(Duff & McMillan, 1951).   Certainly it is true
that anoxaemia or damage to the arterial wall
predisposes to cholesterol deposition and the
development of atherosclerotic lesions (Hueper,
1944, 1945).   Hypercholesterolaemia and in-
stability of the cholesterol-protein attachment
probably result in the accumulation of cholesterol
in the intima but are certainly not the sole
causes of such deposition;   local and haemodynamic
factors may be of equal importance.

4./

4.   Abnormal metabolism of lipids and atherosclerotic lesions in the coronary arteries might both be secondary to some other alteration of physiological function.   It is possible that the abnormalities in the circulating lipids and lipoproteins, the thrombotic tendency and the atherosclerotic lesions in coronary arteries all have a common denominator.   This postulate will now be examined (see Part III) and a hypothesis will be developed that an alteration of normal endocrine balance might act as a common aetiological factor.

PART III

ENDOCRINE IMBALANCE AS AN AETIOLOGICAL FACTOR

A.   THE INFLUENCE OF THE ENDOCRINE GLANDS
     AND ADMINISTERED HORMONES ON THE CIRCULATING
     LIPIDS AND LIPOPROTEINS.

  1.   The circulating lipids and lipoproteins in
       relation to certain physiological endocrine
       changes.

    a.   Menstrual cycle.

        Depression of the plasma cholesterol, the

C/P ratio and the β-lipoprotein cholesterol with

elevation of the α-lipoprotein cholesterol

occurred regularly at ovulation and during

menstruation of 24 young healthy women; and

the opposite changes in the circulating lipids and

lipoproteins developed during the luteal phase

of the cycle (see Page 58 & Figs. 8, 9 and 11).

The secretion of oestrogens also undergoes

regular cyclical variation and is generally

believed to be maximal immediately before ovul-

ation (Smith & Smith, 1936; Brown, 1955) and to

remain high throughout the luteal phase, and some

workers have observed a secondary peak just before

menstruation, (Gustavson et al, 1938).   Progest-

erone secretion, as measured by urinary preg-

nanediol excretion, increases after ovulation to

reach a peak at about the 22nd day of a 28 day

cycle and thereafter gradually falls (Venning &

Brown, 1937). It seems reasonable, therefore, to

correlate the cyclical changes in the circulating

lipids and lipoproteins with the regular varia-

tions in these sex hormones; it is suggested that

the/

the decrease observed in the former at ovulation could result from the increased endogenous secretion of oestrogens and that the opposite changes observed during the luteal phase might possibly be related to the endogenous secretion of progestins.   However, since there is some retention of sodium chloride and water at the time of ovulation and particularly just before menstruation (Thorn et al, 1938), the possibility cannot be entirely excluded that the observed cyclical changes may be partly the result of dilution of the plasma, even although there were no regular changes in the plasma proteins in this series of 24 women.

   b.   Pregnancy.

   Elevation of the plasma cholesterol, the C/P ratio and the β -lipoprotein cholesterol with depression of the α -lipoprotein cholesterol has been observed during pregnancy in 12 young healthy primigravidae (see Page 60 and Figs. 12 and 13).   This hypercholesterolaemia in late pregnancy has been variously ascribed to de-creased elimination of cholesterol in the bile, to increased adrenal synthesis of cholesterol, to placental toxins, to the influence of the foetus and to the metabolic requirements of lactation, but the evidence to implicate any of these/

these factors is circumstantial and unconvincing.
Endocrine influences have also been suggested
as a cause of the hypercholesterolaemia (Schiller,
1919;   Boyd, 1934) and certainly in pregnancy
there are considerable changes from the normal
endocrine balance.

The behaviour of the circulating lipids
and lipoproteins during pregnancy must be con-
sidered in relation to some of these endocrine
changes.   The concentration of oestrogens,
estimated by semiquantitative measurements of
urinary oestrogens, rises steadily during preg-
nancy until the last month when there is probably
no further increase, and at term there is a sharp
drop (Venning, 1946).   This increase in the
urinary concentration of oestrogens (which pre-
sumably reflects the concentration of circulating
oestrogens) is in the region of a thousandfold,
and its occurrence in association with elevation
of the circulating lipids and lipoproteins is at
variance with the suggestion that the endogenous
secretion of oestrogens may cause depression of
these values at ovulation (see Page 92) and with
the observation that the administration of
oestrogen/

oestrogen preparations results in depression of
the circulating lipids and lipoproteins (see Page
119).   It is possible that this lipid-depressant
action of oestrogens is antagonised during preg-
nancy, or that the concentration or relative
proportions of the various oestrogens are such
that the depressant action is lost. The concen-
tration of circulating progesterone, as judged
by urinary pregnanediol excretion, is increased
twentyfold (Venning, 1946), but administered
progesterone does not cause any very striking
elevation of the circulating lipids and lipo-
proteins (see Page 122).   While androgens un-
doubtedly elevate these values (see Page 122)
there is no evidence that androgens are secreted
in excess by pregnant women, and furthermore,
the 17-ketosteroid excretion is not increased
(Venning, 1946).

In pregnancy there is elevation of the
serum protein-bound iodine (Heinemann et al, 1948),
increased radio-active iodine uptake (Pochin, 1952),
and during the last 4 months elevation of the basal
metabolic rate (Javert, 1940).   The hypercholest-
erolaemia of pregnancy seems anomalous in the
presence of these signs of increased thyrotrophic
and/

and thyroid activity, which usually result in depression of the circulating lipids and lipo-proteins (see Page 106). Adrenocortical activity, as measured by determination of the blood and urinary 17-hydroxycorticoids, is also increased during pregnancy (Venning, 1946; Gemzell, 1953), yet the administration of adrenocorticotrophic hormone and cortisone cause depression of the circulating lipids and lipoproteins (see Page 109).

The development of hypercholesterolaemia in the presence of increased oestrogenic, thyroid, and adrenocortical activity is unexpected and at present unexplained. There is evidence that the secretion of growth hormone from the hyperplastic anterior pituitary is, in common with the secretion of the other trophic hormones, increased during pregnancy (Young, 1951). Moreover, increased secretion of growth hormone may be partly res-ponsible for the increased insulin requirement of the diabetic pregnant woman and the birth of large babies from prediabetic and diabetic mothers (Young, 1953). It is possible that more complete understanding of the interrelationship of growth hormone, glucagon and insulin may help to explain the hypercholesterolaemia of pregnancy.

c./

c. Menopause.

Elevation of the plasma cholesterol and
C/P ratio has been observed during the first
postmenopausal decade (Fig. 14 and Table VIII).
This might be related to the decrease in oestro-
genic activity and also to any/postmenopausal
decrease in thyroid or adrenocortical activity.
If there is a hormonal basis for the postmeno-
pausal rise in healthy women, it does not appear
to influence hypercholesterolaemic subjects in
the same way since no postmenopausal rise was
observed in women with established ischaemic heart
disease (Figs. 17 and 18). Explanation of the
subsequent fall in the circulating lipids is
not readily forthcoming; this fall has recently
been confirmed by Adlersberg et al (1956), who
observed the postmenopausal rise only between the
ages of 53 and 57.

2. Incidence of coronary disease in certain
   endocrine disorders.

a. Diabetes mellitus.

The occurence of coronary disease in a
diabetic patient was first reported in 1864 by
Seegen; subsequently it has been indicated that
coronary/

98.

coronary atherosclerosis and ischaemic heart
disease are common in diabetic patients (Joslin,
1930; Blotner, 1930). Advanced and often
occlusive atherosclerosis of the coronary arteries
has been demonstrated at autopsy in 53% of 973
diabetic patients in contrast to 28% of 5829 non-
diabetic patients of comparable age; and similarly,
coronary thrombosis was observed at autopsy in
32% of 790 diabetic patients and in 16% of 5650
nondiabetic patients of comparable age (Liebow and
Hillerstein, 1949). Thus coronary disease is
twice as common in diabetic than in nondiabetic
subjects. Provided they have the disease long
enough, diabetics will almost invariably become
atherosclerotic; of 484 autopsies on patients
who had had diabetes for 5 or more years, only 4
were free from atherosclerosis (Warren, 1938).
Diabetes also hastens the development of coronary
atherosclerosis and ischaemic heart disease in
youth (Joslin and Wilson, 1950) and eliminates the
relative immunity of women (Clawson & Bell, 1949).

    b. Myxoedema.

    In 1888 a Report by the Committee of the
Clinical Society of London described atheromatous
changes in the arteries of only 5 out of 84 cases
of myxoedema, but since then there have been fre-
quent reports/

reports of a high incidence of coronary athero-
sclerosis in association with myxoedema (Black
and Kampmeier, 1934; Bruger and Rosenkrantz,
1942; Means, 1948). However, the existence of
this association is not universally accepted, and
Andrus (1953) does not believe that there is
any conclusive evidence that gross atherosclerosis
is an inevitable consequence of hypothyroidism.
Myxoedema has its highest incidence at ages when
atherosclerosis is most common, and it is often
difficult therefore to assess the influence that
myxoedema may have had on the development of
coronary atherosclerosis. The report of the 1888
Committee is often quoted against any correlation
between myxoedema and atherosclerosis, but it
should be noted that the occurrence of athero-
sclerosis in only 6% of the cases is much less than
would be expected in autopsies of non-myxoedematous
adult subjects (White et al, 1950), and the methods
of assessment of atherosclerotic lesions were not
very comprehensive. However, the observation of
a high incidence of atherosclerosis in patients
with endemic cretinism and goitre (Wegelin, 1925),
and in young patients with thyroid deficiency
(Fishberg, 1924; Hoelzer, 1940) offers more
substantial/

substantial support for a relation between the
two conditions.

The incidence of ischaemic heart disease
may not be increased in <u>untreated</u> myxoedema
(Means, 1948). Although it is probable that
myxoedema favours the development of coronary
atherosclerosis, the function of these diseased
arteries may be quite adequate for the demands
of the myocardium when metabolism is retarded.
As soon as metabolism is increased by the
administration of a thyroid derivative, the
atherosclerotic coronary arteries become relatively
inadequate and angina develops; and there is no
doubt that the development of angina in <u>treated</u>
myxoedematous patients is common (Means, 1948).
Like diabetes, myxoedema may diminish the sex
difference in the incidence of ischaemic heart
disease; in a group of 59 cases of spontaneous
myxoedema, ischaemic heart disease occurred as
frequently in women under 50 as in men under 50
(Bartels & Bell, 1939). Endocrine disorders
occurred in 30% of women who developed ischaemic
heart disease under the age of 40 (Table XV).
Table XV./

Table XV.   An analysis of the health of 20 women
            who developed ischaemic heart disease
            before the age of 40.

Associated disease or disorder	Number of patients
Endocrine disorders:	
Myxoedema	2
Menopause aged 32	1
Ovariectomy, aged 22	1
Diabetes mellitus	1
Cushing's disease	1
Hypertension	5
During pregnancy or puerperium	4
Anaemia	1
No associated disease	4

    c.   Hyperadrenocorticism (Cushing's Disease).

        Atherosclerosis was observed in 3 out of
9 autopsied cases originally described by Cushing
(1932), and extensive calcification was apparent
radiologically in the aorta of his first case;
all 4 were under the age of 28 years.   Generalised
vascular sclerosis has been reported in 6 patients
who died from Cushing's disease or hyperadreno-
corticism, and cholesterol deposits have been
observed in the endothelium of small arteries
(Heinbecker, 1944);   5 of these 6 patients died
under the age of 33 years.   Subsequently, the
statement that atherosclerosis, especially of the
coronary/

coronary arteries, occurs with increased frequency
in Cushing's disease has often been made without
detailed evidence to support this contention
(Williams, 1951; Wakerlin, 1952; Katz & Stamler,
1953); however, Thompson and Eisenhardt (1943)
reported 98 cases, of whom 63 had an autopsy,
without mention of atherosclerosis. Cushing's
disease is associated very freqeuntly with
hypertension and diabetes and often with depression
of thyroid function, and any premature development
or increase in the incidence of atherosclerosis
may well be related to these other influences.
The suggestion that atherosclerosis develops
earlier and to a greater degree in Cushing's
disease is not well documented or controlled,
and the evidence that it results from hyperadreno-
corticism is largely circumstantial.

    d. Castration.

    Examination of the hearts of 49 women, in
whom bilateral ovariectomy was performed between
the ages of 2 and 42 years, revealed more severe
coronary atherosclerosis, decade for decade, than
the average degree of coronary atherosclerosis
of a control group (Wuest et al, 1953); yet only
3/

3 of these 49 patients were found to have had
clinical manifestations of coronary disease.   No
other comparable investigation has yet been re-
ported and no studies of the hearts of male
castrates have been recorded.

 3. The circulating lipids and lipoproteins in
   certain endocrine disorders.

 a. Diabetes mellitus.

  There is little doubt that the frequency
and severity of atherosclerosis in diabetes can
be correlated with the duration of uncontrolled
glycosuria and hyperglycaemia and with the con-
comitant hyperlipaemia and hypercholesterolaemia
(Joslin et al, 1946).   Hyperlipaemia is more
marked than hypercholesterolaemia (Hirsch et al,
1953), yet of a series of 273 diabetic patients
50% exhibited elevated circulating lipids while
92% of those with severe atherosclerosis showed
hyperlipaemia (Pomeranze and Kunkel, 1950).
Hypercholesterolaemia was often observed in young
diabetics (Rabinowitch, 1935).   Ultracentrifuge
studies in diabetics have shown abnormally high
concentrations of the particular cholesterol-con-
taining lipoproteins (S_f 12-20 class) which are
associated with atherosclerosis (Gofman et al,
1950).   The β-lipoprotein cholesterol is higher in
diabetics with atherosclerosis/

atherosclerosis than in diabetics without athero-
sclerosis, and higher in the latter group than
in nondiabetics (Baker et al, 1955).   Nearly
all the diabetic patients seen in the Department
of Cardiology had co-existent vascular disease,
and no comprehensive analysis has been made in
diabetics without vascular disease; however,
Table XVI demonstrates the degree of abnormality
of the circulating lipids and lipoproteins in a
man of 19, in whom diabetes presented by the
development of diffuse eruptive cutaneous xanthomata.

Table XVI.   The circulating lipids in a 19 year old
man with xanthoma diabeticorum.
Insulin was started on 1.8.55.

	Plasma cholesterol mg%	C/P Ratio	Comments
1.8.1955	1230	1.71	Diffuse xanthomatous eruption and very lipaemic.
3.8.1955	720	1.21	Lipaemic
6.8.1955	172	0.78	Clear plasma

Diabetes is, therefore, associated with
abnormal circulating lipids and lipoproteins and
a parallel relationship exists between hyper-
lipaemia and hyperglycaemia (Hirsch et al, 1953).
These/

These abnormalities of the circulating lipids and lipoproteins are probably a direct consequence of insulin lack, but they may also depend partly on the degree of renal damage associated with intercapillary glomerulo-sclerosis; tubular insufficiency and proteinuria in nephrosis is associated with both hyperlipaemia and hyper-cholesterolaemia (Peters & Man, 1943c).

    b. <u>Myxoedema</u>.

      It is well known that myxoedema is associated with hypercholesterolaemia (Mason <u>et</u> <u>al</u>, 1930; Hurxthal, 1934; Gildea <u>et</u> <u>al</u>, 1939). This increase in circulating cholesterol is not due sole-ly to lowered metabolism, since dinitro-0-cresol will restore metabolism to normal without reducing the hypercholesterolaemia associated with hypo-thyroidism (Dodds & Robertson, 1933) and reduction in metabolism in euthyroid subjects is not accompanied by hypercholesterolaemia (Mason <u>et</u> <u>al</u>, 1930). In myxoedema the concentration of cholesterol on the β-lipoprotein fraction is elevated (Malmros & Swahn, 1953), and ultra-centrifuge studies have indicated that the con-centration of the potentially atherogenic (S_f 12-20) class of lipoproteins is also increased (Gofman <u>et</u> <u>al</u>, 1950).

    c./

c. Thyrotoxicosis.

It is well established that the circulating
cholesterol is low when there is hyperfunction of
the thyroid (Epstein & Lande, 1922; Mason et al,
1930; Peters & Man, 1943). The plasma cholester-
ol is generally lowest in patients with very
marked hyperthyroidism, but there is no absolute
direct correlation with the height of the basal
metabolic rate.

d. Hyperadrenocorticism (Cushing's Disease).

Analyses of the circulating lipids in
hyperadrenocorticism have not been reported
frequently. The blood cholesterol was reported
in 5 of Cushing's cases and was elevated in one
(Cushing, 1932); there appears to be no consis-
tent abnormality, although there is occasionally
hypercholesterolaemia (Heinbecker, 1944;
Heinbecker & Pfeiffenberger, 1950) but hyper-
cholesterolaemia has also been reported in
Addison's disease (Medvei, 1935).   Peters and
Van Slyke (1946) believe that any hypercholesterol-
aemia, which may occur in association with hyper-
adrenocorticism, can be explained by accompanying
diabetes and renal damage.   Thus, there is no
convincing evidence that Cushing's disease is
associated with hypercholesterolaemia or with an
increased incidence of atherosclerosis and
ischaemic heart disease (Page 101).

e./

e.   <u>Castration.</u>

No study of the circulating lipids and
lipoproteins has not yet been reported after
gonadectomy, but in 2 out of 3 patients, in whom
bilateral ovariectomy had been carried out, there
was elevation of the circulating lipids and
lipoproteins (Table XVII);   angina occurred in
1 woman at the age of 38 and a myocardial infarct
developed in another at the age of 49.   In the
third patient, there was auricular fibrillation
and mild thyrotoxicosis, which might account
for the normal circulating lipid levels.

<u>Table XVII.</u>   The circulating lipids and lipo-
proteins in 3 castrated women.

	Plasma cholest- erol mg%	C/P ratio	$\alpha:\beta$ lipo- protein ratio	Comments
1. Aged 38, Ovariec- tomy, aged 22	288	1.96	9:91	Angina
2. Aged 49. Ovariec- tomy, aged 27	577	1.56	4:96	Myocardial infarct
3. Aged 52. Ovariec- tomy, aged 31	201	0.87	–	Auricular fibrilla- tion and mild thy- rotoxicos- is

4./

4. The influence of hormones on the circulating
    lipids and lipoproteins.

        The effect of extraneous influences on the
circulating cholesterol, such as a dietary con-
straint or the administration of hormones, may be
delayed for several days.   The "half-life" of the
circulating cholesterol is 8 days (London and
Rittenberg, 1950), and thus any change in the
circulating cholesterol or the concentration of
cholesterol on the β -lipoprotein fraction re-
sulting from an alteration in the rate of bio-
synthesis or degradation will be delayed.   However,
when there is a redistribution of cholesterol
between the cells and extracellular fluid (such
as may occur after the administration of certain
thyroxine analogues), the effect on the circulating
cholesterol could be apparent within a day or two.

        In general the subjects of these investi-
gations were either healthy men, or hypercholest-
erolaemic men with ischaemic heart disease and
electrocardiographic evidence of a myocardial
infarct.   It was not considered justifiable to
administer hormones with a pronounced metabolic
effect, such as ACTH, cortisone and the sex
hormones, to healthy subjects.

    a./

a. Pituitary hormones.

i. Adrenocorticotrophic hormone.

The daily intramuscular administration of
50 mg. adrenocorticotrophic hormone (ACTH) for 8
days resulted in significant depression of the
plasma cholesterol, the C/P ratio and the β-lipo-
protein cholesterol with elevation of the α-lipo-
protein cholesterol in 6 hypercholesterolaemic men
with proven coronary disease (Table XVIII). The
daily administration of 100 mgm. of ACTH for 5 days
resulted in similar changes in the circulating
lipids and lipoproteins in a further 6 hyper-
cholesterolaemic men with proven coronary disease
(Fig. 21 and Table XVIII) (Oliver & Boyd, 1955c,
1956b). These results confirm the experiences
of Conn et al, (1950) in 3 normal men but appear
to contradict the observations of Adlersberg
et al, (1950, 1951); this apparent discrepancy
will be considered later (see section concerning
cortisone on page 111).

ii. Thyroid stimulating hormone.

The daily intramuscular administration
of 10 units of thyroid stimulating hormone (TSH)
for 4 days did not cause any significant change
in the circulating lipids and lipoproteins in
3 subjects with primary myxoedema (Table XVIII)
(Oliver and Boyd, 1956b). The/

Table XVIII. The effect of pituitary hormones on the circulating lipids and lipoproteins in hypercholesterolaemic men.

	Number of subjects	Plasma cholesterol mg%	C/P ratio	β-lipoprotein cholesterol	α-lipoprotein cholesterol
Adrenocorticotrophin: (intramuscularly)					
50 mg. daily for 8 days	6	-15%	-5%	-22%	+16%
100 mg. daily for 5 days	6	-16%	-16%	-30%	+57%
Thyroid-stimulating hormone: (intramuscularly)					
10 units daily for 4 days to hypothyroid men	3	No significant change			
10 units daily for 4 days to euthyroid men	3	-28%	-16%	-21%	+24%
Growth hormone: (intravenously)					
200 mg. over 30 minutes to healthy men	3	No significant change			

Fig. 21. The changes in the circulating lipids
          and lipoproteins during the intramusc-
          ular administration of 100 mg of ACTH
          daily to 6 hypercholesterolaemic men
          with ischaemic heart disease.

The intramuscular administration of the same dose of TSH for 4 days to 3 hypercholesterolaemic men with coronary disease resulted in significant depression of the circulating lipids and lipo- proteins (Table XVIII).   These results suggest that TSH has no direct action on the circulating lipids and lipoproteins, and that any effect in euthyroid subjects is mediated through stimulation of functioning thyroid tissue.

iii.   Growth hormone.

The intravenous infusion over a period of 30 minutes of 180 ml. of normal saline con- taining 200 mg. of a porcine growth hormone (STH) prepartion did not result in any immediate or delayed change in the circulating lipids and lipoproteins in 3 healthy men (Table XVIII). This same growth hormone preparation was known to be effective in rats in stimulating hepatic cholesterol biosynthesis after inhibition by hypophysectomy.   It is unlikely, therefore, that the preparation was metabolically inert, although it is possible that the dose was too small to produce any effect on the circulating lipids and lipoproteins;   this may be so, but it has been shown that a similar dose resulted in a fall in blood/

blood aminoacid nitrogen and hyperglycaemia
(Carballeira et al, 1952).   It is possible that
prolonged intramuscular administration of a crys-
talline growth hormone preparation (not widely
available) or of a human growth hormone prepara-
tion might influence the circulating lipids and
lipoproteins, although in general the metabolic
activity of growth hormone preparations is dis-
appointing at present.

    iv. Gonadotrophic hormones

       Preparations of gonadotrophic hormones,
whether predominantly follicular-stimulating or
luteinising, have not been administered in man
with the object of assessing their influence on
the circulating lipids and lipoproteins.   Animal
assays have indicated that these preparations
do not have either a purely follicular-stimulating
or a purely luteinising action, and thus it was
considered that study of the influence of the
individual sex hormones on the circulating lipids
and lipoproteins would be of greater value.

    b. Adrenal cortical hormones

      i. Cortisone

       The daily oral administration of 100 mg.
of/

of cortisone acetate for 10 days resulted in significant depression of the plasma cholesterol, the C/P ratio and the β-lipoprotein cholesterol with elevation of the α-lipoprotein cholesterol in 6 hypercholesterolaemic men with proven coronary disease (Fig. 22 and Table XIX).   The daily administration of 150 mg. and of 200 mg. of cortisone acetate each for 10 days resulted in similar changes in the circulating lipids and lipoproteins in two further groups of 6 hyper-cholesterolaemic men with proven coronary disease (Table XIX) (Oliver and Boyd, 1955c, 1956b).

The influence of cortisone acetate on the circulating lipids and lipoproteins is, therfore, similar to that of ACTH.   These results in hypercholesterolaemic subjects, all of whom were at work and reasonably healthy, do not necessarily contradict those of Adlersberg et al (1950, 1951), who showed that ACTH and cortisone raised the serum cholesterol in patients who were severely ill with diffuse lupus erythematosis, polyarteritis nodosa, dematomyositis and other collagen diseases.   In their cases the sympto-matic improvement, the reduction of fever, and the/

Fig. 22. The changes in the circulating lipids and lipoproteins during the oral administration of 100 mg of cortisone acetate daily to 6 hypercholesterolaemic men with ischaemic heart disease.
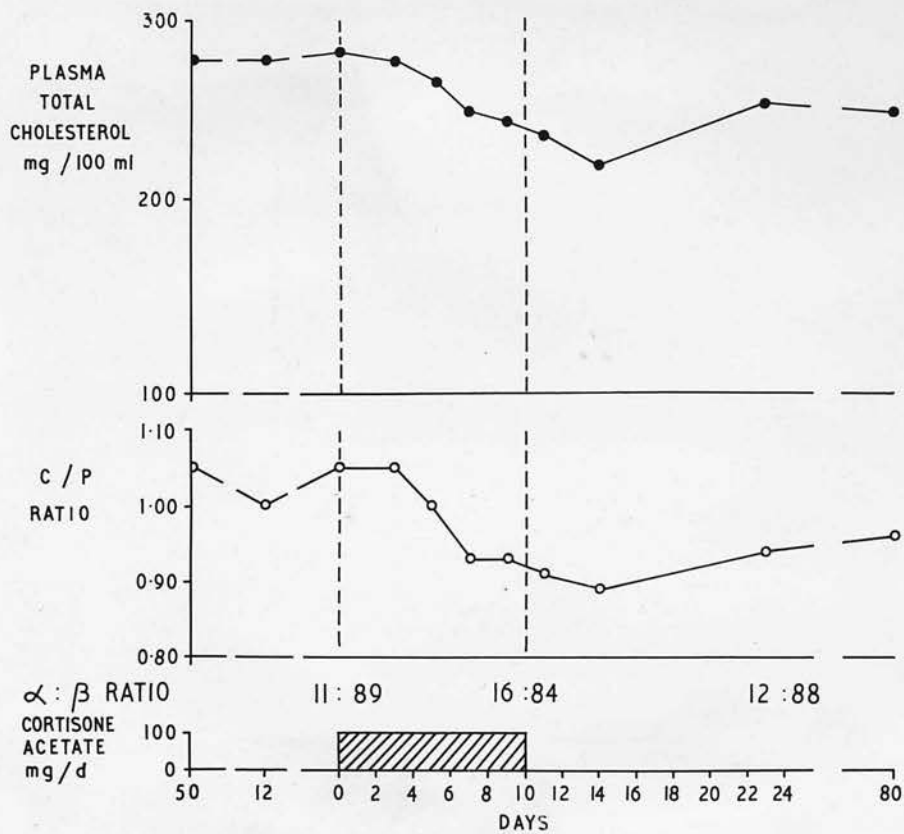
Table XIX. The effect of cortisone acetate and desoxycorticosterone acetate on the circulating lipids and lipoproteins in hypercholesterol-aemic men with coronary disease.

	Number of subjects	Plasma cholest-erol mg%	C/P ratio	β-lipo-protein cholesterol	α-lipo-protein cholesterol
Cortisone acetate: (orally)					
100 mg daily for 10 days	6	-12%	-9%	-16%	+17%
150 mg daily for 10 days	6	-15%	-11%	-17%	+11%
200 mg daily for 10 days	6	-13%	-2%	-28%	+47%
Desoxycorticosterone acetate: (intramuscularly)					
5 mg daily for 5 days	6	-6%	-4%	-10%	+48%

the return of appetite probably contributed to the rise in circulating cholesterol; moreover, in the few of their patients who were hyper-cholesterolaemic and who had some disease other than a collagen disorder, the plasma cholesterol was not significantly raised by ACTH and cortisone. Mann and White (1953) considered that any eleva-tion of the plasma cholesterol produced by ACTH and cortisone could be due to relief of the acutely stressful phase of the disease in these very ill patients, thus permitting the low cholesterol levels to return to normal; certain-ly in Adlersberg's cases the initial levels of circulating cholesterol were unusually low in contrast to the values obtained in healthy American adults.

The response of the circulating lipids and lipoproteins to ACTH and cortisone in the 30 men referred to in Tables XVIII and XIX was remarkably uniform, and it is believed that, although these men were initially hypercholest-erolaemic, depression of these values may be the true response to ACTH and cortisone.

ii. Desoxycorticosterone acetate./

ii. Desoxycorticosterone acetate.

Although desoxycorticosterone acetate
(DOCA) is probably not a natural secretion of the
adrenal cortex, it has been included in this
section on the assumption that its effect on the
circulating lipids and lipoproteins will represent
the effect of naturally-occurring mineralocorti-
coids. The daily intramuscular administration of
5 mg. of desoxycorticosterone acetate for 5 days
resulted in slight depression of the plasma
cholesterol, the C/P ratio and the β-lipoprotein
cholesterol with increase in the α-lipoprotein
cholesterol in 6 hypercholesterolaemic men with
proven coronary disease (Table XIX); the packed
cell volume was reduced by 6%.

c. Thyroid hormones

It has been known for many years that
thyroid extract lowers the plasma cholesterol
and phospholipids in hypothyroid and in euthyroid
subjects, and at the same time increases their
metabolic requirements (Lévy & Lévy, 1931;
Turner & Steiner, 1939; Gildea et al, 1939);
and more recently it has been shown that thyroxine
also reduces the β-lipoproteins (Malmros & Swahn,
1953) and/

and lowers the concentration of the "atherogenic group" of low density lipoproteins (S_f 12-20) as determined by the ultracentrifuge (Strisower et al, 1954). The principle hormones secreted by the thyroid are thyroxine and triiodothyronine, which is derived from thyroxine by deiodination and probably has similar physiological actions.

    i. ℓ-thyroxine sodium.

    Hypothyroid subjects. The daily oral administration of 300 μg. of ℓ-thyroxine sodium for 8 weeks resulted in significant depression of the plasma cholesterol, C/P ratio and the β-lipoprotein cholesterol with elevation of α-lipoprotein cholesterol in 6 patients with classical myxoedema (Fig. 23 and Table XX). The mean weight loss was 7 lbs. and the basal metabolic rate was increased by 17%.

    Euthyroid subjects. The daily oral administration of 600 μg. of ℓ-thyroxine sodium for 2 weeks resulted in similar changes in a further 6 euthyroid hypercholesterolaemic men with proven coronary disease (Fig. 24 and Table XXI), except that the concentration of cholesterol on the α-lipoprotein fraction also fell. The mean weight loss after 3 weeks was 4 lbs. and the basal metabolic rate increased by 13%.

    ii./

Fig. 23. The changes in the circulating lipids and lipoproteins, and in the basal metabolic rate and weight, during the oral administration of 300 μg. of ℓ-thyroxine sodium daily to 6 patients with myxoedema.

Table XX.   The effect of thyroxine and some analogues on the circulating lipids and lipoproteins in hypercholesterolaemic hypothyroid patients.

	Number of subjects	Plasma cholesterol mg%	C/P ratio	β-lipoprotein cholesterol	α-lipoprotein cholesterol
l-thyroxine sodium: 300 μg. daily for 8 weeks	6	-40%	-6%	-29%	+42%
l-triiodothyronine: 30 μg. daily for 10 days	2	-22%	-7%	-18%	+31%
Triiodothyroacetic acid: 0.5 mg. daily for 17 days	1	-31%	-10%	-40%	+38%
5 mg. daily for 6 days	2	-37%	-23%	-26%	+35%

Fig. 24. The changes in the circulating lipids and lipoproteins and in weight during the oral administration of 600 μg. of l-thyroxine sodium to 6 euthyroid hypercholesterolaemic men with ischaemic heart disease.
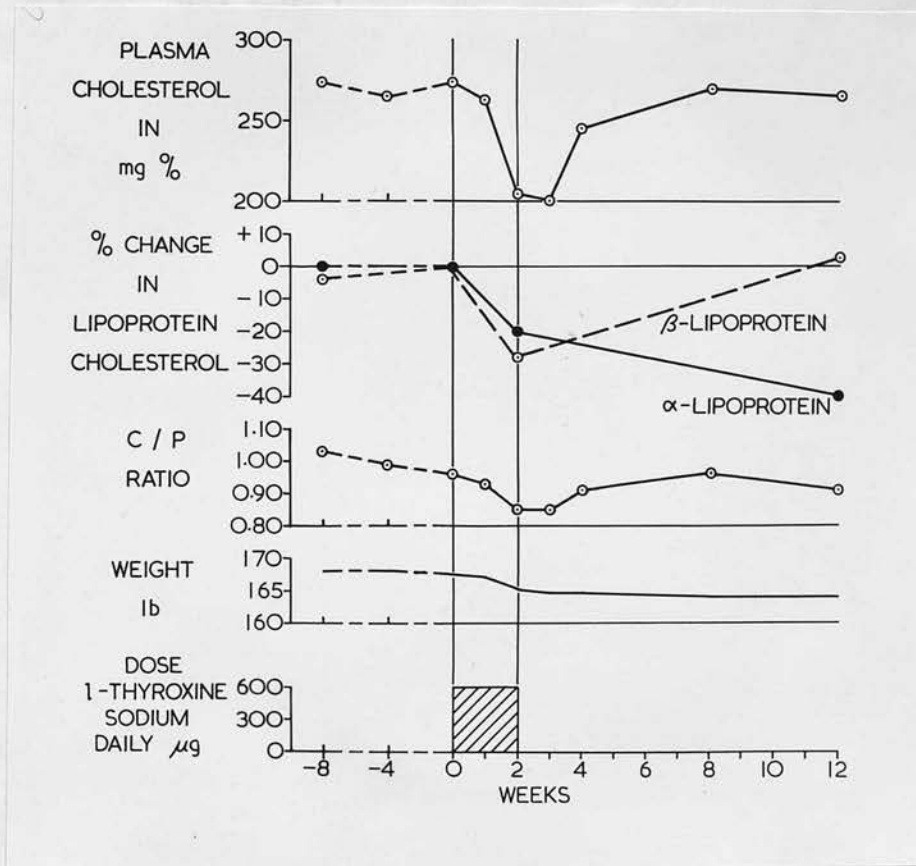
Table XXI. The effect of thyroxine and some analogues on the circulating lipids and lipoproteins in hypercholesterolaemic euthyroid men with coronary disease.

	Number of subjects	Plasma cholesterol mg%	C/P ratio	β-lipoprotein cholesterol	α-lipoprotein cholesterol
ℓ-thyroxine sodium: 600 μg. daily for 2 weeks	6	-27%	-17%	-29%	-20%
ℓ-triiodothyronine: 40-60 μg. daily over 8 weeks	3	-16%	-14%	-27%	+42%
† 40-100 μg. daily over 7 weeks	3	-34%	-17%	-36%	-12%
Triiodothyroacetic acid: † 0.5-4 mg. daily over 12 weeks	6	-21%	-12%	-26%	+45%

† No elevation of basal metabolic rate.

ii. l-triiodothyronine.

Hypothyroid subjects. The daily oral administration of 30 μg. of l-triiodothyronine for 10 days resulted in depression of the plasma cholesterol, the C/P ratio and the β-lipoprotein cholesterol with elevation of the α-lipoprotein cholesterol in 2 patients with classical myxoedema. The mean weight loss was 6 lbs. and the basal metabolic rate increased by 15% (Table XX).

Euthyroid subjects. The daily oral administration of 40 μg. of l-triiodothyronine for 5 weeks and then 60 μg. for 2 weeks to 3 euthyroid hypercholesterolaemic men with proven coronary disease resulted in significant depression of the plasma cholesterol, the C/P ratio, and the β-lipoprotein cholesterol with elevation of the α-lipoprotein cholesterol (Table XXI). There was no change in weight or in the basal metabolic rate.

The daily oral administration of a larger dose (40 μg. for 2 weeks and then 100 μg. for 4 weeks) of l-triiodothyronine resulted in similar changes in the circulating lipids and lipoproteins, except that the concentration of cholesterol/

cholesterol on the α-lipoprotein fraction also
fell, in a further 3 euthyroid hypercholesterol-
aemic men with proven coronary disease (Fig. 25
and Table XXI).   The mean weight loss was 6 lbs.
and the basal metabolic rate increased by 11%.

    iii, Triiodothyroacetic acid.

       Triiodothyronine can undergo oxidative
deamination to triiodothyropyruric acid with
subsequent oxidative decarboxylation of the latter
compound to triiodothyroacetic acid (triac);
this acetic acid analogue of triiodothyronine
was first synthesised by Pitt-Rivers (1953).

       Hypothyroid subjects. The daily oral
administration of 0.5 mg. of triac for 21 days
resulted in significant depression of the plasma
cholesterol, the C/P ratio and β-lipoprotein
cholesterol with elevation of the plasma cholest-
erol in a 69 year old woman with classical
myxoedema (Fig. 26 and Table XX).   There was no
weight loss and no increase in the basal metabolic
rate.   The daily oral administration of a larger
dose (4 mg. for 6 days) of triac resulted in
similar changes in the circulating lipids and
lipoproteins in 2 patients with classical myxoedema
(Fig. 27 and Table XX).   Within 24 hours there
was/

Fig. 25.　The changes in the circulating lipids
and lipoproteins, and in the basal meta-
bolic rate and weight, during the oral
administration of 40 and 100 μg. of l-
triiodothyronine daily to 3 euthyroid
hypercholesterolaemic men with ischaemic
heart disease.

Fig. 26. The changes in the circulating lipids and lipoproteins, and in the basal metabolic rate and weight, during the oral administration of 500 μg. of triiodothyroacetic acid daily to a 69 year old woman with myxoedema.

Fig. 27.   The changes in the circulating lipids
            and lipoproteins, and in the basal meta-
            bolic rate and weight, during the oral
            administration of 4 mg of triiodothyro-
            acetic acid daily to 2 patients with
            myxoedema.
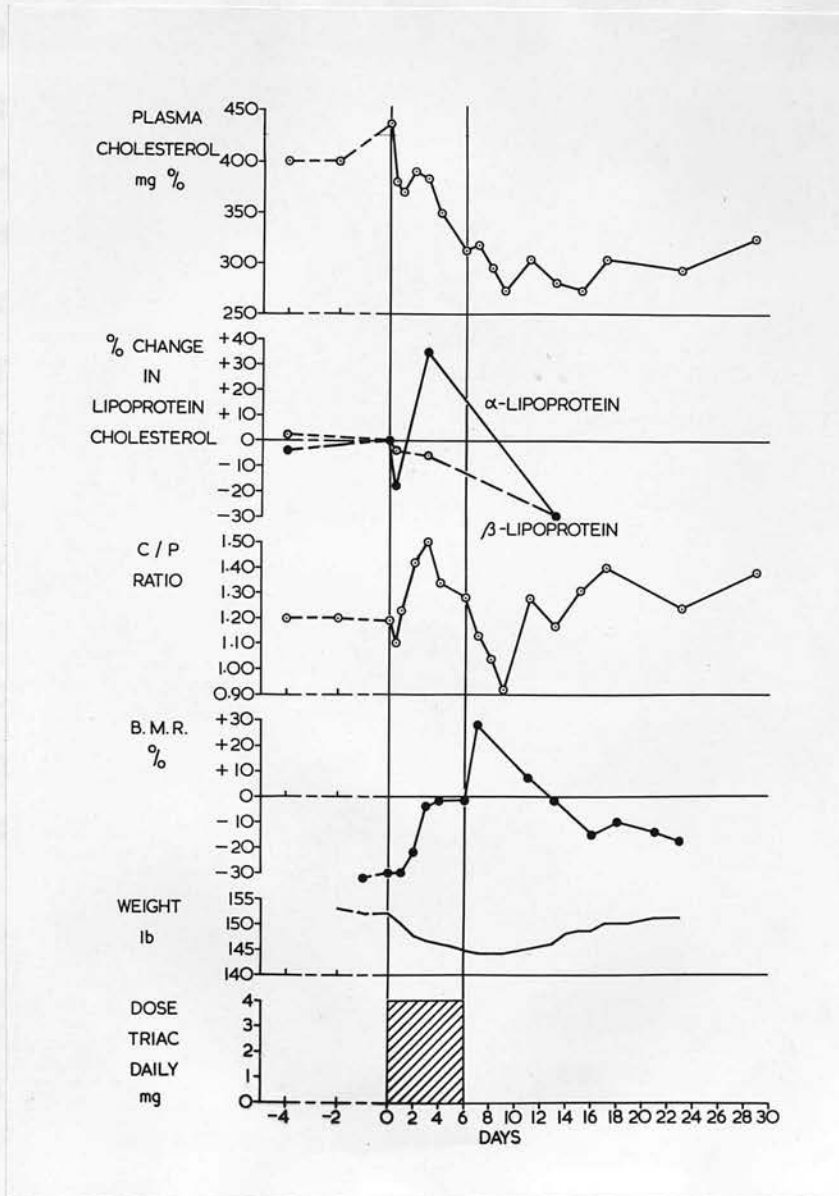
was improvement of the clinical signs of myxoedema, weight loss and an increase in the basal metabolic rate.

Euthyroid subjects. The daily oral administration of increasing doses (from 0.5 mg. to 4 mg.) of triac over a period of 95 days resulted eventually in depression of the plasma cholesterol, the C/P ratio and the β-lipoprotein cholesterol with elevation of the α-lipoprotein cholesterol in 6 euthyroid hypercholesterolaemic men with proven coronary disease; there was no significant effect until 3 mg. of triac were administered daily (Fig. 28 and Table XXI). There was no weight loss, and the mean basal metabolic rate was not significantly altered although it was elevated in two of the men by 13% and 20% respectively. The action of some of these thyroid hormones has been described recently (Oliver & Boyd, 1957, Boyd and Oliver, 1957), and their potential therapeutic application will be considered later (see Page 153).

    d. Sex hormones

      i. Oestrogens
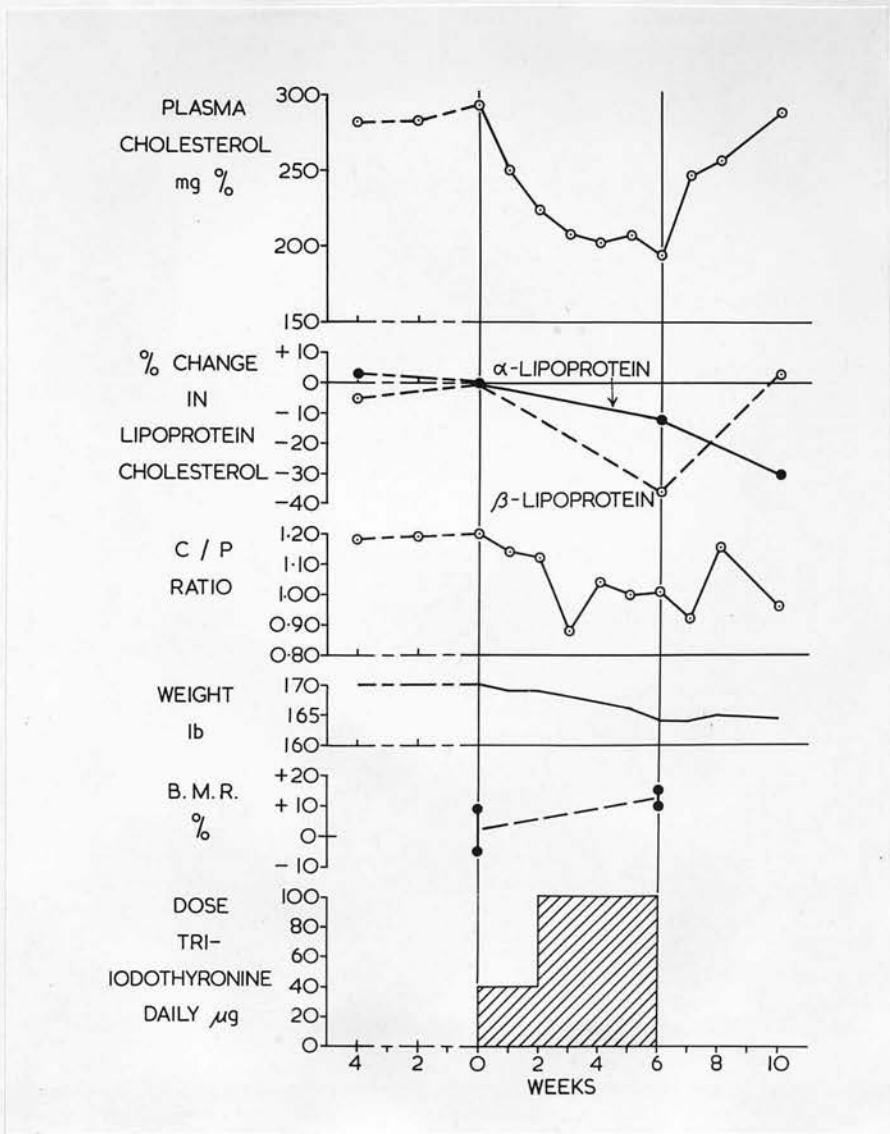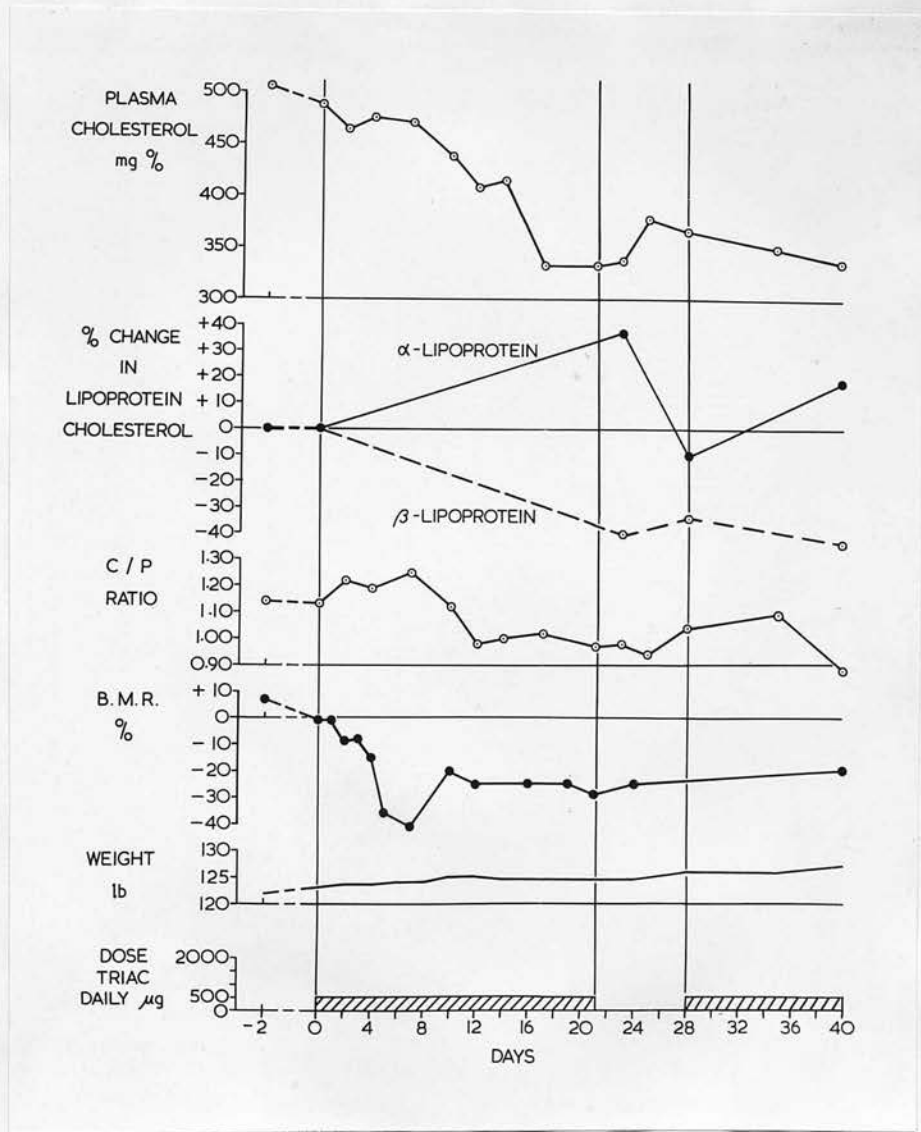
The suggestion that depression of the circulating/

<u>Fig. 28.</u> The changes in the circulating lipids and lipoproteins, and in the basal metabolic rate and weight, during the oral administration of increasing doses of triiodothyroacetic acid daily to euthyroid hypercholesterolaemic men with ischaemic heart disease.
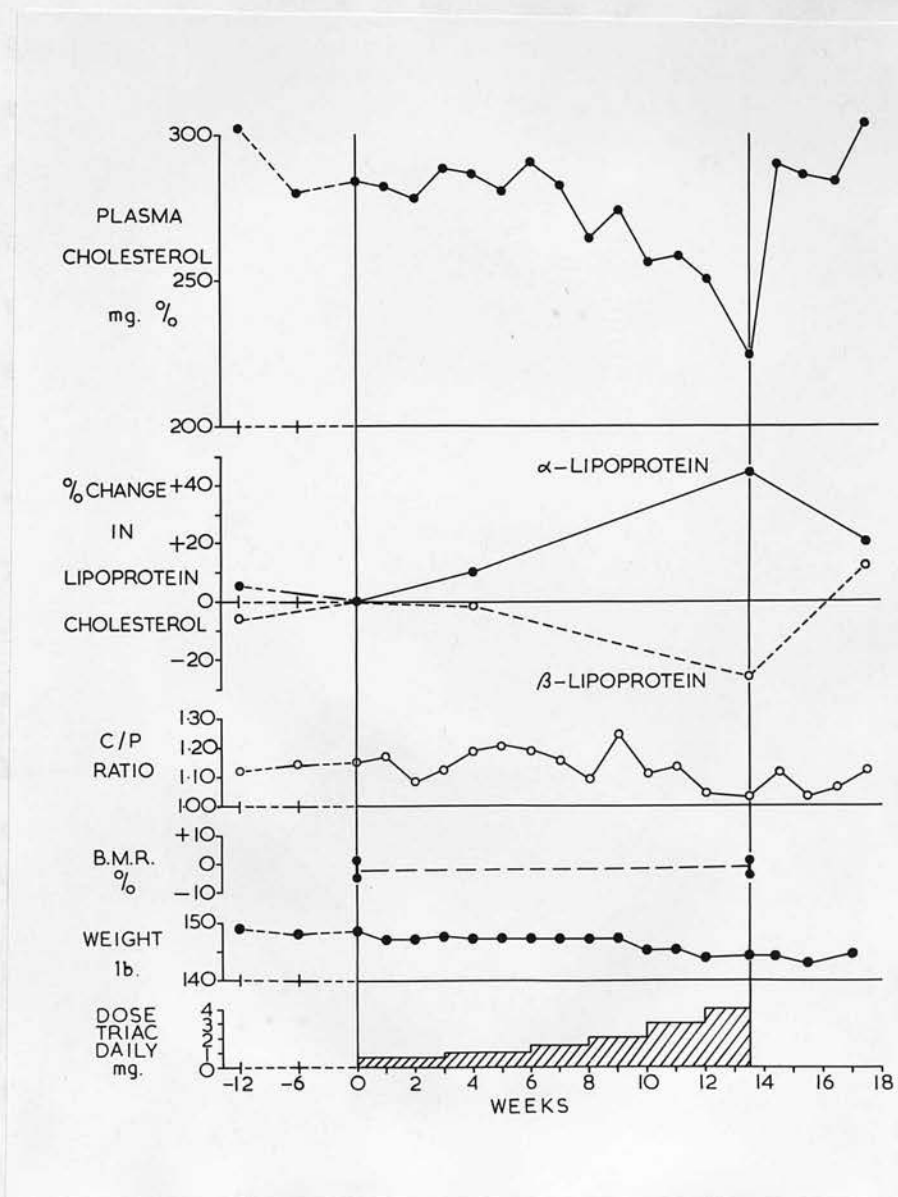
circulating cholesterol, the C/P ratio and
β-lipoprotein cholesterol at ovulation (see
Page 92) might be due to endogenous secretion
of oestrogens indicated that the influence of
administered oestrogens on the circulating
lipids and lipoproteins required assessment.

(1) Ethinyl oestradiol.

Pilot study.   As the circulating lipids
had not previously been studied after the
administration of oestrogens, a small pilot study
was necessary in order to determine the minimal
dose required to alter these values.   After a
control period of 7 weeks, ethinyl oestradiol
was administered orally to 3 hypercholesterol-
aemic men with proven coronary disease and the
dose was gradually increased over the next 10
weeks from 0.02 mg. to 0.4 mg. daily.   The mean
plasma cholesterol remained constant up to a
dose of 0.2 mg. of ethinyl oestradiol, but there-
after decreased significantly (Fig. 29).   This
preliminary study suggested that the effective
oral dose of ethinyl oestradiol would not be
less than 0.2 mg. daily. (Oliver and Boyd, 1954).

Graduated study.   The daily oral
administration of increasing doses from 0.2 mg.
to/

Fig. 29. The changes in the circulating lipids during a pilot study of the oral administration of ethinyl oestradiol to 3 hypercholesterolaemic men with ischaemic heart disease.
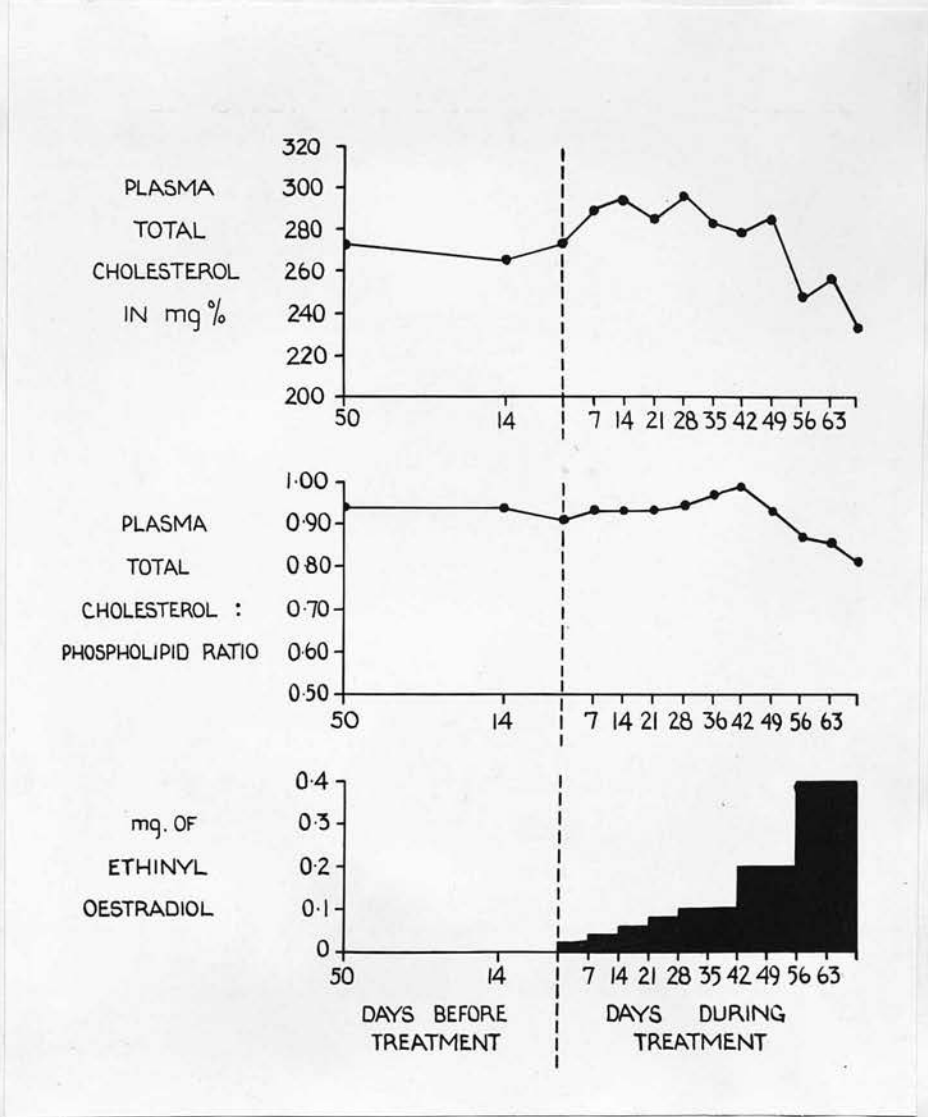
to 1 mg. of ethinyl oestradiol over a period of
12 weeks to 15 hypercholesterolaemic men with
proven coronary disease resulted in significant
depression of the plasma cholesterol and C/P
ratio (Fig. 30 and Table XXII).   Ethinyl
oestradiol and other oestrogens decrease the
β -lipoprotein cholesterol and increase the
α -lipoprotein cholesterol (Fig. 31).

Large dose study.   The daily oral
administration of 1 mg. of ethinyl oestradiol
for 6 weeks to 30 hypercholesterolaemic men with
proven coronary disease resulted in similar changes
in the circulating lipids and lipoproteins
(Fig. 32 and Table XXII).

Further experience concerning the
administration of ethinyl oestradiol to hyper-
cholesterolaemic men with coronary disease will
be considered in the section concerning thera-
peutic implications (see Page 158).

(2)   Hexoestrol & Stilboestrol.

The daily oral administration of 60 mg.
of hexoestrol for 14 days to 6 hypercholesterol-
aemic men with proven coronary disease also
resulted in significant depression of the plasma
cholesterol/

Fig. 30.   The changes in the circulating lipids
            during the oral administration of
            increasing doses of ethinyl oestradiol
            to 15 hypercholesterolaemic men with
   ischaemic heart disease.

Table XXII. The effect of oestrogens on the circulating lipids and lipoproteins in hypercholesterolaemic men with coronary disease.

	Number of subjects	Plasma cholesterol mg%	C/P ratio	β-lipoprotein cholesterol	α-lipoprotein cholesterol
Ethinyl oestradiol:					
Increasing dose to 1 mg.	15	-23%	-33%	-30%	+61%
1 mg. daily for 6 weeks.	30	-16%	-23%	-20%	+29%
Hexoestrol:					
60 mg. daily for 2 weeks.	6	-9%	-16%	-13%	+30%
Stilboestrol:					
5 mg. daily for 4 weeks.	3	-11%	-10%	-12%	+21%
Oestradiol:					
12 mg. daily for 2 weeks.	3	-8%	-8%	-19%	+15%
24 mg. daily for 2 weeks.	3	-12%	-8%	-16%	+19%
Oestrone:					
30 mg. daily for 2 weeks.	3	-3%	-3%	-2%	+14%
50 mg. daily for 2 weeks.	3	-9%	0	-6%	+20%
Oestriol (intramuscularly):					
200 mg. daily for 1 week.	2	-25%	-6%	-30%	+71%

Fig. 31.   The changes in the circulating lipo-

protein cholesterol during the oral

administration of ethinyl oestradiol

to hypercholesterolaemic men with

ischaemic heart disease.

(The serum is applied to the filter
paper at the point represented by the
vertical line and is induced to migrate
towards the anode represented by the +).

CHOLESTEROL ATTACHED TO THE β AND α LIPOPROTEINS BEFORE
AND DURING OESTROGEN THERAPY IN HYPERCHOLESTEROLAEMIC
MALES WITH CORONARY SCLEROSIS AS DETERMINED BY FILTER
PAPER ELECTROPHORESIS

Fig. 32.   The changes in the circulating lipids
           during the oral administration of 1 mg
           of ethinyl oestradiol daily to 30 hyper-
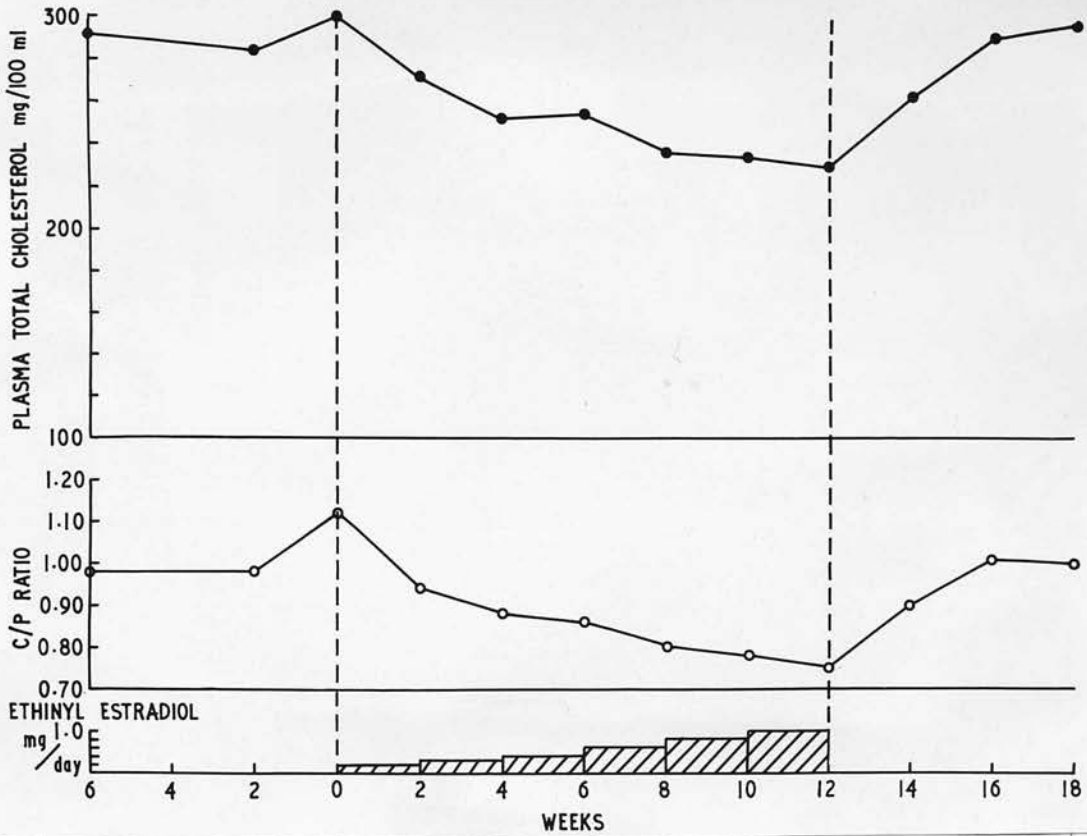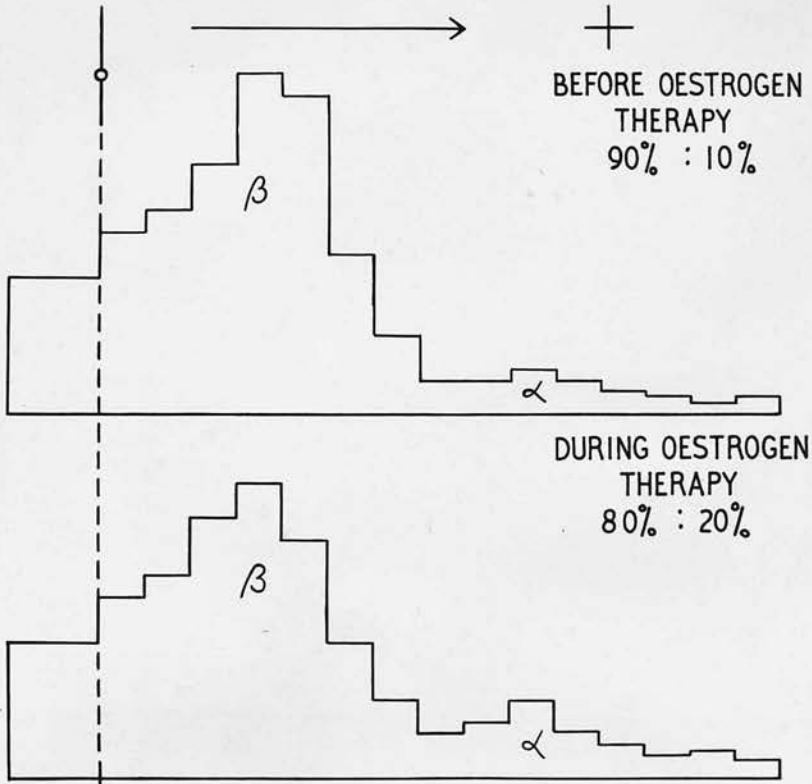   cholesterolaemic men with ischaemic
           heart disease.
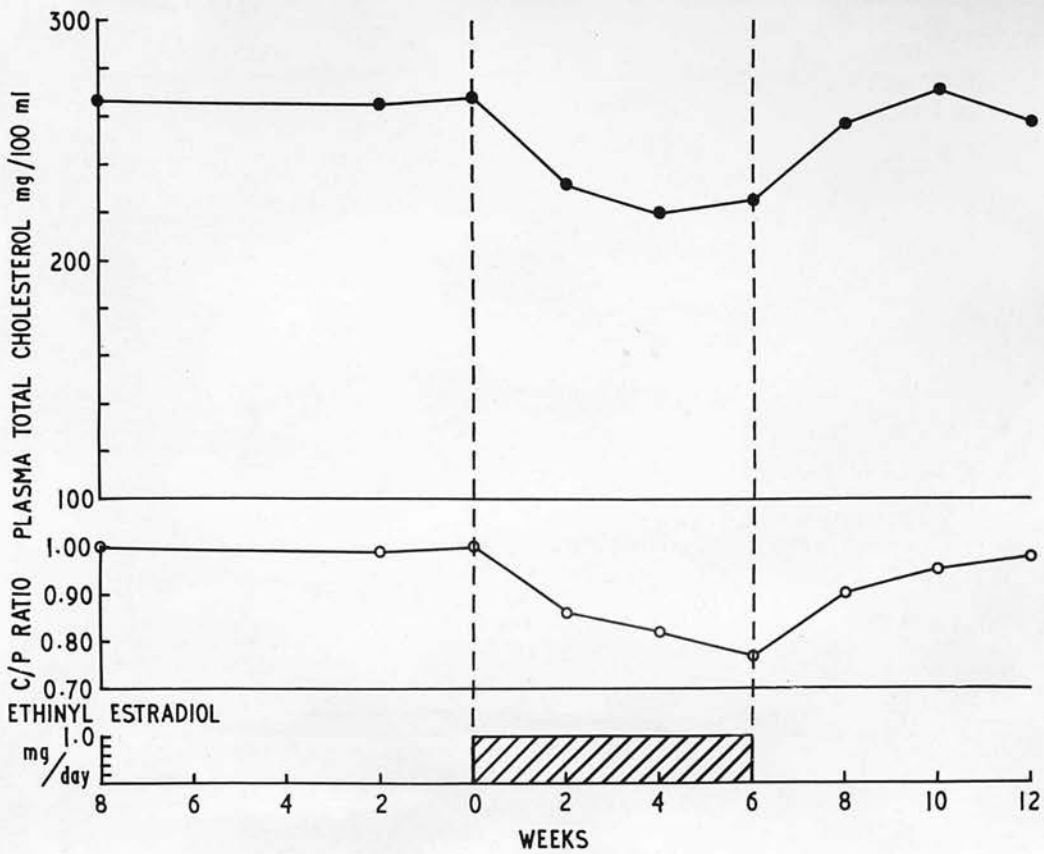
cholesterol, C/P ratio and β-lipoprotein cholesterol with elevation of the α-lipoprotein cholesterol (Table XXII); the administration of 5 mg. of stilboestrol for 4 weeks to 3 hypercholesterolaemic men with coronary disease produced comparable results (Table XXII).

(3) Naturally occurring oestrogens.

The administration of oestradiol, oestrone or oestriol to 15 hypercholesterolaemic men with coronary disease resulted in similar depression of the plasma cholesterol, the C/P ratio and the β-lipoprotein cholesterol with elevation of the α-lipoprotein cholesterol (see Table XXII).

ii. Progesterone

The daily intramuscular administration of 100 mg. of progesterone for 5 days to 6 hypercholesterolaemic men with proven coronary disease resulted in slight but probably insignificat elevation of the circulating lipids and lipoproteins (Table XXIII).

iii. Androgen

The daily sublingual administration of 50 mg. of methyl testosterone for 10 days to 6 hypercholesterolaemic men with coronary disease resulted in/

Table XXIII. The effect of progesterone and methyl testosterone on the circulating lipids and lipoproteins in hyper-cholesterolaemic men with coronary disease.

	Number of subjects	Plasma cholesterol mg%	C/P ratio	β-lipoprotein cholesterol	α-lipoprotein cholesterol
Progesterone (intramuscularly)					
100 mg. daily for 5 days	6	+5%	+8%	+3%	+27%
Methyl testosterone					
50 mg. daily for 10 days	6	+30%	-4%	+11%	-55%
20 mg. daily for 4 weeks after ethinyl oestradiol administration	12	+50%	+43%	+56%	-26%

in elevation of the plasma cholesterol and β-lipoprotein cholesterol with depression of the α-lipoprotein cholesterol (Fig. 33 and Table XXIII). This action of methyl testosterone was even more pronounced when administered immediately after a course of ethinyl oestradiol (Fig. 43 and Table XXIII).

The influence of some of these sex hormones on the circulating lipids and lipoproteins in men with ischaemic heart disease has been reported recently (Oliver and Boyd, 1956a, 1956b).

e. Pancreatic hormones.

i. Glucagon.

The intravenous infusion of 2 mg. of glucagon ("hyperglycaemic factor of the pancreas") over a period of 30 minutes to 3 healthy men resulted in immediate elevation of the blood glucose and 5 days later slight elevation of the plasma cholesterol and β-lipoprotein cholesterol with depression of the α-lipoprotein cholesterol (Fig. 34 and Table XXIV) (Oliver and Boyd, 1956b).

ii. Insulin.

The daily subcutaneous administration of 50 units of insulin for a period of 5 weeks to 6 men, who were suffering from depressive states (in Jordanburn Nerve Hospital), but were otherwise healthy, did not result in any significant change in the circulating lipids and lipoproteins. (Table XXIII)/

Fig. 33. The changes in the circulating lipids
and lipoproteins during the oral admin-
istration of 50 mg of methyl testosterone
daily to 6 hypercholesterolaemic men
with ischaemic heart disease.
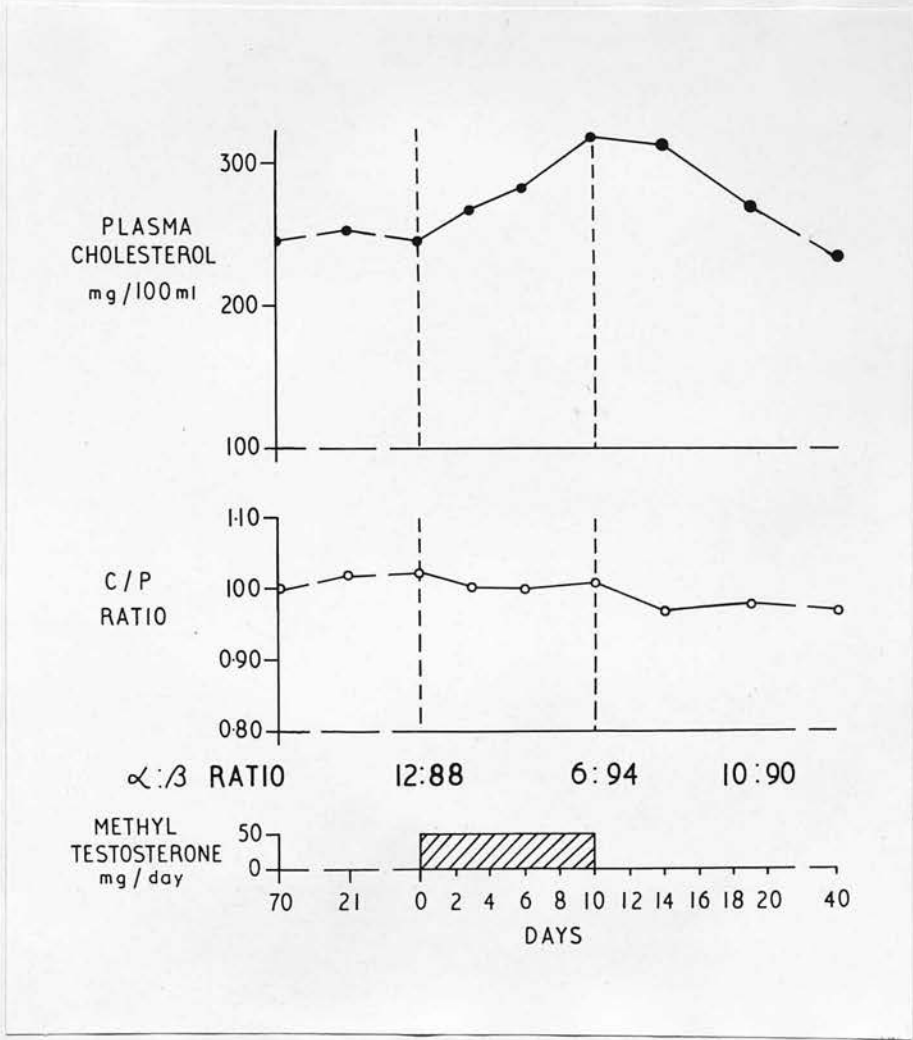
Fig. 34.   The changes in the plasma cholesterol
           and the blood glucose following the
           intravenous infusion of 2 mg. of glucagon
           in 180 ml. of isotonic saline to 3
           healthy men.
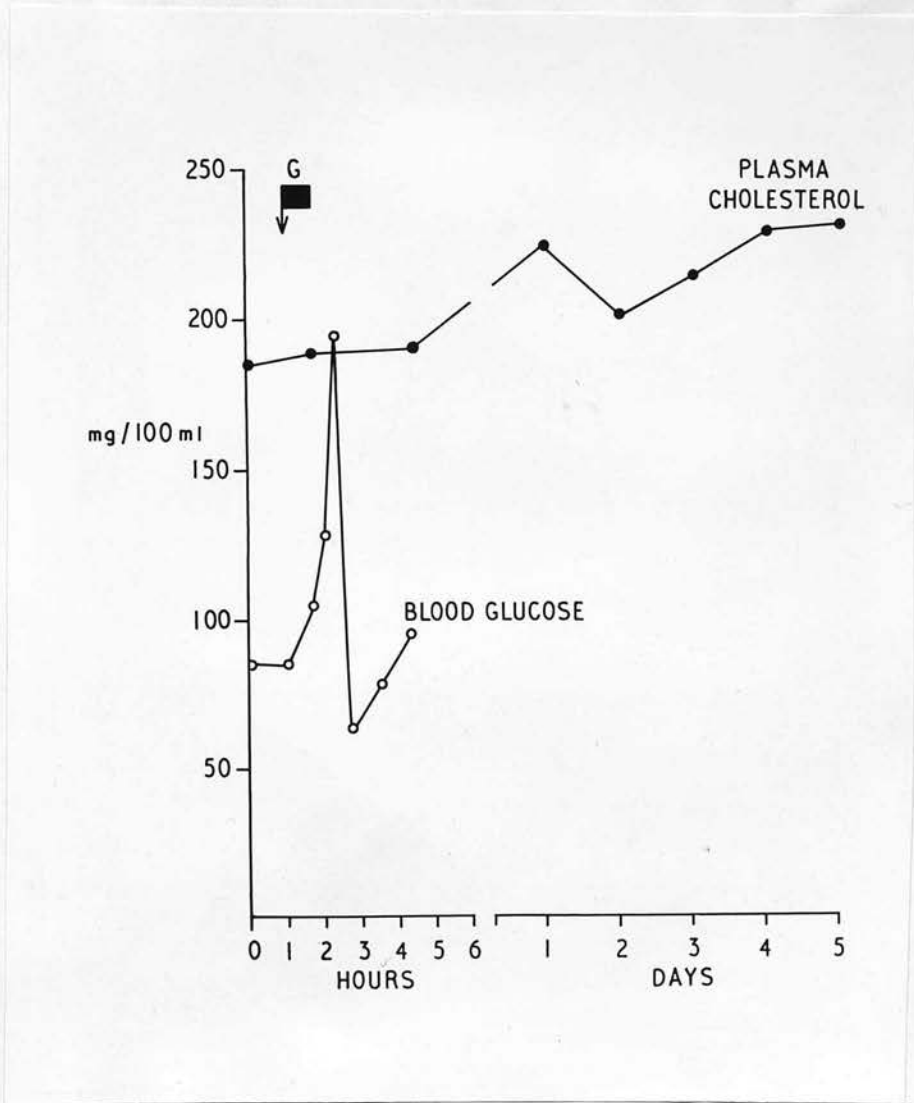
Table XXIV. The effect of glucagon and insulin on the circulating lipids and lipoproteins in healthy and diabetic men.

	Number of subjects	Plasma cholesterol mg%	C/P ratio	β-lipoprotein cholesterol	α-lipoprotein cholesterol
Glucagon (intravenously):					
Effect of 2 mg. 6 days later	3	+10%	+2%	+13%	-10%
Insulin (subcutaneously):					
50 units daily for 4 weeks	3	No	significant	change	
32 units daily for 6 weeks (average dose)	6	-48%	-12%	-26%	+52%

(Table XXIII), although the induced hypo-
glycaemia stimulated the patients to eat and
they increased in weight by an average of 10 lbs.

The daily subcutaneous administration of
insulin to 6 hypercholesterolaemic diabetic
patients resulted in significant depression of
the plasma cholesterol, the C/P ratio and β-
lipoprotein cholesterol with an increase in α-
lipoprotein cholesterol; these changes occurred
in parallel with the fall in blood sugar
(Table XXIV).

B.   THE INFLUENCE OF HORMONES ON THE DEVELOPMENT
     OF CORONARY ATHEROSCLEROSIS AND ISCHAEMIC
     HEART DISEASE.

In the belief that abnormal cholesterol
metabolism, a thrombotic tendency and local
haemodynamic disturbances in the arterial wall
all favour the development of coronary athero-
sclerosis and ischaemic heart disease, the
influence of hormones on cholesterol metabolism,
the fluid state of the blood and the arterial wall
will now be considered.

1.   The influence of hormones on cholesterol
     metabolism.

It has already been indicated that the
sex/

125.

sex hormones probably exert a physiological
influence on the circulating lipids and lipo-
proteins. It has also been emphasised that in
patients with certain endocrine disorders, such
as diabetes and myxoedema, there is an unsually
high incidence of coronary atherosclerosis and
clinical coronary disease. Furthermore, in
patients with these same endocrine disorders,
yet without any features of coronary disease,
analysis of the circulating lipids and lipoproteins
frequently reveals the abnormal patterns assoc-
iated with overt ischaemic heart disease. The
administration of certain hormones has resulted
in alterations in the circulating lipids and
lipoproteins, and these hormonal influences
are summarised in Fig. 35. Administration of
adrenal, thyroid and oestrogen preparations
reduces the circulating cholesterol if it is high,
and administration of androgens, and possibly
also of glucagon and progesterone, raises the
circulating cholesterol above normal levels.
There is, then, little doubt that the endocrine
glands and hormones can influence cholesterol
metabolism.

2./

HYPERCHOLESTEROLAEMIA

Adrenal +   Androgens +

Thyroid +   ? Glucagon +

Oestrogens +   ? Progesterone +

NORMOCHOLESTEROLAEMIA
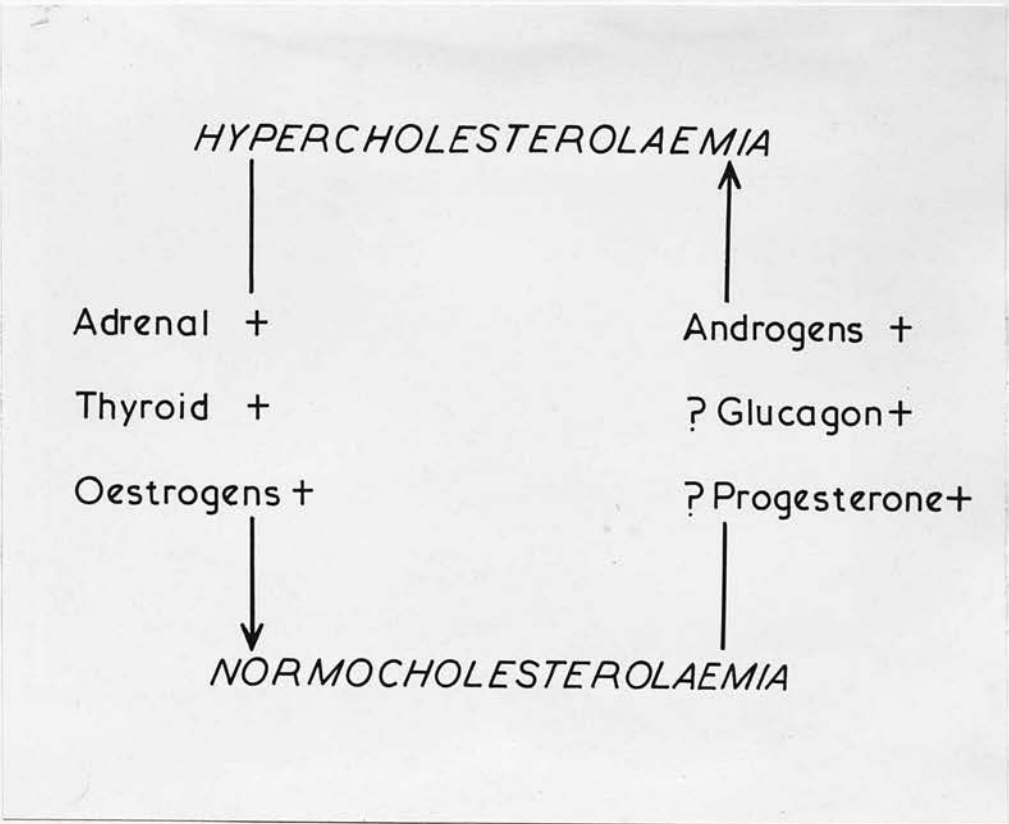
2. The influence of hormones on the
    fluid state of the blood.

  a. Fibrinogenesis.

       The coagulation system may reasonably
be regarded as consisting of two opposing groups
of dynamic processes: those which favour the
formation of thrombin and fibrin, such as Factors
V and VII, calcium ions and other activators
and accelerators;   and those which prevail against
the formation of thrombin and fibrin, such as
heparin, antithromboplastin and other inhibitors,
    those
or/which dispose of formed fibrin, such as the
fibrinolytic system.   Both these groups of
processes can be influenced in vivo by hormones,
although there is no evidence that the incidence
of thrombosis is increased in endocrine disorders.

       Adrenaline and experimentally-induced
stress accelerate clotting (Mills et al, 1928),
increase prothrombin concentration (Uvnas, 1942;
Leibetseder, 1951), and decrease heparin activity
(Constantini and Tintori, 1951;   Donzelot and
Kaufmann, 1952).   The administration of ACTH
and cortisone during treatment of various
collagen diseases has been associated with
shortening of the clotting time and of the
heparin-/

heparin-retarded clotting time (Cosgriff et al, 1950), and an increase in the incidence of thromboembolic episodes in such patients (Cosgriff, 1951). In certain animals shortening of the bleeding time followed experimental stress ACTH and cortisone, yet similar stress failed to influence the bleeding time after hypophysectomy or after adrenalectomy (Ungar, 1945), these observations suggest that the pituitary and adrenal cortex can both influence bleeding time; however, in man cortisone shortens prolonged bleeding time (Jacobson, 1953). The clotting mechanism may be partly under neurogenic influence as adrenergic stimuli increase and cholinergic stimuli decrease the tendency to thrombosis (de Takats, 1944); it may be that post-operative thrombosis is an extreme example of this increase in haemostasis following stress. The administration of growth hormone to dogs decreased the clotting time and prothrombin time (Campbell, 1953).

b. Fibrinolysis

Indirect testing of the efficiency of the fibrinolytic system by the intravenous administration of thrombin to rats suggested that ACTH and/

and cortisone inhibit fibrinolytic activity,
and that growth hormone, TSH, desoxycorticosterone
acetate and testosterone increase fibrinolytic
activity (Ungar, 1954). Stefanini and Damashek
(1955) have confirmed that ACTH and cortisone
decrease fibrinolysis, yet experimental stress
and adrenaline increase fibrinolytic activity
(Macfarlane & Biggs, 1946; Biggs et al, 1947).

3. The influence of hormones on the arterial
   wall.

Any agent which increases the hyperton-
icity of arteries by producing contraction of
the muscular elements in the arterial wall re-
duces the vascular bed and necessitates a com-
pensatory acceleration of blood flow and an
increase in dynamic pressure. Moreover, pro-
longed and inordinate vasoconstriction results
in compression of the vasa vasorum by the con-
tracting media with ischaemia of the arterial
wall, particularly the inner layers, and the
increased density of the contracted arterial wall
may also hinder the normal diffusion of tissue
fluids. Compression or active constriction of
the capillaries within the arterial wall can
result in local anoxaemia, which will alter
the/

the normal tissue metabolism and may act as an nidus for accumulation of fat and lead ultimately to an atheromatous lesion.

a. Vasomotor tone.

Prolonged arterial hypertonicity can lead to clinical hypertension, and the positive correlation between hypertension and coronary disease, particularly in women, is well known (Davis and Klainer, 1940; Master, 1953). Certain hormones increase vasomotor tone and, although it is probable that the hypertension of Cushing's disease is largely related to the associated renal disease rather than the excessive secretion of adrenal steroids, one of the secretions of the adrenal cortex probably has a definite vasopressor action. Desoxy-corticosterone can reasonably be regarded as a synthetic substitute for part of the adrenal cortical secretions, and experimentally it and the natural mineralocorticoid, 17-desoxocortisone, induce hypertension and hyaline changes in the arterial wall (Selye, 1950); however, aldosterone is said to lower vascular tone (Mach and Fabre, 1955). In view of the vasodilating action of oestrogens,/

oestrogens, their withdrawal at the menopause has been implicated as a cause for the not uncommon development of hypertension and peripheral vascular insufficiency at the menopause (Scherf, 1940).   Adrenaline and noradrenaline cause elevation of the blood pressure through their powerful vasoconstricting action; vaso-constriction occurs in the renal, splanchnic and cerebral arteries, but vasodilation takes place in the coronary arteries.   In 1903 Josué pro-duced degenerative lesions in the aortae of rabbits after repeated injections of adrenaline; many investigators have confirmed this observation and similar but more chronic arterial lesions, principally fibrous in character, develop in the coronary arteries as well as other arteries after the administration of small daily doses of adrenaline (Lange, 1924).

b. Capillaries.

Whereas adrenaline increases vasomotor tone, arterial lesions may be partly due to constriction of the capillaries supplying the arterial wall. An increase in capillary resis-tance occurs after the application of various forms of stress and after the administration of ACTH/

131.

ACTH and insulin, and it has been suggested that
this increase in capillary resistance may be due
to stimulation of adrenocortical activity
(Robson & Duthie, 1950). Adrenalectomy in rats
is followed by reduction of capillary resistance,
which can subsequently be restored by cortisone
(Kramar and Simy-Kramar, 1953). ACTH and
cortisone also decrease capillary permeability
and fibroblast activity, and thereby impair
organisation of intravascular thrombi and minor
injuries of the arterial intima (Payling-Wright,
1954; Wang et al, 1955). Capillary permeability
is increased in myxoedema and thyroxine therapy
restores it rapidly to normal (Lange, 1944).
In addition to producing changes in the arterial
wall it has long been observed that myxoedema
is associated with marked degenerative changes
in the muscle fibres of the myocardium (Ord, 1880).

C. CONCLUSION.

  1. The main proposition of this thesis.

      The association of abnormalities of
cholesterol metabolism, the coagulation system
and the arterial wall with the development of
coronary/

coronary atherosclerosis and ischaemic heart disease has already been emphasised. Frank endocrine diseases and the administration of hormones can influence cholesterol metabolism, the fluid state of the blood and metabolism in the arterial wall, and it is postulated that the normal homeostasis of these three processes could be disturbed by alteration of the physiological endocrine balance. The thesis is proposed, therefore, that an endocrine imbalance could contribute to the development of coronary atherosclerosis and ischaemic heart disease.

The principle weakness of this thesis lies in the fact that no marked and consistent abnormality of endocrine gland function has yet been found in patients who have the clinical features of coronary disease. It is unlikely that there is any gross endocrine disturbance in coronary atherogenesis as this would have wide metabolic repercussions and would have been recognised years ago, and it is possible that a comparatively minor disturbance may have passed largely unnoticed due to the crudeness of the available methods for assessing the function of the endocrine glands. However, there is some suggestive evidence that endocrine function may not be entirely normal in patients with ischaemic heart disease.

The/

The basal metabolic rate was lower than
normal in a group of patients who developed
ischaemic heart disease under the age of 40, and
it was concluded that their thyroid function might
be slightly depressed (Lerman & White, 1946);   this
observation has received confirmation recently
(Gertler et al, 1953), but there was no significant
difference in radioactive iodine excretion
between coronary patients and euthyroid subjects.
The concentration of circulating protein-bound
iodine decreases with advancing age (Perlmutter
& Riggs, 1949), but no clear correlation has been
found between protein-bound iodine levels and
coronary disease (Tucker & Keys, 1951) although
Marmorston et al (1955) found a greater depression
of circulating protein-bound iodine in post-
menopausal women with myocardial infarcts when
compared with healthy postmenopausal controls.

The relative freedom of premenopausal
women from ischaemic heart disease is probably
related to the endocrine differences between the
sexes (see Pages 37, 92), and it is interesting to
find that 30% of women who developed ischaemic
heart disease before the age of 40 had some
endocrine/

endocrine disturbance (Table XV). In view of
the sex difference in the incidence of ischaemic
heart disease and the depressant action of
oestrogens on the circulating cholesterol, it
might be expected that women who develop a
myocardial infarct would excrete less oestrogen
than a comparable control group, and biological
determination of urinary oestrogen have indicated
that this is so in two such groups of postmeno-
pausal women (Marmorston et al, 1955). When
Dr. Bauld worked in the Department of Biochemistry
at Edinburgh University, he started to estimate
the urinary excretion of oestrogens in healthy
men and men with ischaemic heart disease. These
investigations (Bauld et al, 1956) suggested that,
in response to an injection of estradiol-17β ,
the ratio of oestriol to oestrone was significantly
elevated in men after myocardial infarction when
contrasted with healthy men, although the total
excretion of oestrogens was unchanged. This
difference was not observed in 3 men, who did not
have a myocardial infarct but were suspected of
having clinical coronary disease. These two
studies suggest that oestrogen metabolism might
be/

135.

be disturbed in some patients with ischaemic
heart disease.   The proneness of males to
ischaemic heart disease has led to an attempt
to assess masculinity in such patients.   Men
who develop ischaemic heart disease under the
age of 40 were considered to be morphologically
more masculine than healthy men, but there was
no consistent or significant difference in the
excretion of 17-ketosteroids nor was there any
correlation between the circulating cholesterol
levels and the 17-ketosteroid excretion
(Gertler et al, 1953).   Blood and urinary
corticoid levels have not been reported in
patients with ischaemic heart disease except
immediately after an acute myocardial infarct.

      Thus endocrine gland function has not
been carefully investigated in patients with
angina and in patients who have recovered from a
myocardial infarct;   and, at present, the
evidence obtained from standard tests of endocrine
function is not adequate to refute or confirm
the thesis that an endocrine imbalance might
contribute to the development of coronary
atherosclerosis.

  2.   <u>The aetiology of ischaemic heart disease</u>.
      By/

By 1955 20% of all the deaths in Great
Britain were due to arteriosclerotic or coronary
heart disease and the crude death rate from
ischaemic heart disease had increased seventyfold
in half as many years, yet before this century
angina was a rarity and coronary thrombosis had
been diagnosed only once during life.   There is
good reason to believe that this remarkable
change in the statistics of the disease represents
a real increase in its prevalence, and attention
must be directed, therefore, towards potentially
deleterious influences which have developed during
this century.   There have been fundamental and
far-reaching social and economic changes.   The
standard of living of the civilised nations has
never been higher and has resulted in an
abundance of food not previously experienced.
Industrialisation and world wars have revolution-
ised all our lives, and have created a new sense
of strain and urgency and new responsibilities
which many find difficult to withstand.

    a. <u>Diet</u>.

    In general, the intake of calories,
protein and fat have increased with the rise in
purchasing power which has followed the improvement
in/

in the standard of living during this century.
It is probably true to say that the physical
activity and energy expenditure of many people
has been reduced. There is now greater energy
intake and less energy expenditure, and if energy
intake exceeds energy expenditure, it is axiomatic
that calories must be stored within the organism.
Conservation of calories is most economically
achieved by storing fat, which is deposited in
many sites probably including the liver and
arteries. When the intake of dietary calories
is frequently in excess of the expenditure of
calories by physical activity, a chronic dis-
turbance of lipid metabolism might result.

In countries where the incidence of
ischaemic heart disease is low, the contribution
of fat to total caloric intake is also low. As
the percentage of calories derived from fat
increases from the poorer to the richer nations
so the incidence of ischaemic heart disease
increases, and elaborate epidemiological
surveys indicate a positive correlation between
the social and geographic incidence of ischaemic
heart disease with the consumption of fat (Keys
and Anderson, 1954; Keys et al, 1955; Bronte-
Stewart et al, 1955). In most civilised
communities more than one-third of the total
caloric/

caloric intake is derived from fat, and it is possible that this habitual daily ingestion of large quantities of fat, particularly if mostly from animal sources, may disturb the homeostasis of lipid metabolism and favour the development of coronary atherosclerosis and of ischaemic heart disease; when the diet contains excessive fat the levels of the circulating lipids and β -lipoproteins rise (Anderson and Keys, 1953; Keys et al, 1955) and there is more frequent and greater development of postprandial lipaemia, which can accelerate blood coagulation (Fullerton et al, 1953; Poole, 1955; Robinson and Poole, 1956) and inhibit fibrinolysis (Greig, 1956).

b. Environmental "stress".

Even in countries where the fat consumption is high, most of the population fortunately does not suffer from ischaemic heart disease; of 5 men, whose environment and responsibilities are apparently closely comparable, only one may be expected to develop ischaemic heart disease. There is, then, an individual susceptibility, which is not well understood, but may be partly determined by heredity. The incidence of ischaemic heart disease/

disease is greatest in executive, professional, business and intellectual occupations, where heavy responsibilities often demand exacting and prolonged mental work (Ryle & Russell, 1949; Registrar-General for Scotland 1949-1953; Report of Oslo Life Insurance Companies, 1956) (Fig. 36). The psychological strain and emotional tensions that often accompany these responsibilities are undoubtedly stressful to some people, particularly if they have inherited an ambitious and con-scientious personality, whereas others appear to be able to adapt themselves without undue difficulty. For example, the failure of certain inadequate personalities to adapt themselves to the environment can result in excessive appetite and disturbance of lipid metabolism with subsequent obesity (Hamburger, 1951; Brosin, 1954); this psychological production of obesity is probably mediated through the hypothalamus, which plays an important role in the control of appetite (Clark et al, 1938; Hetherington and Ranson, 1940). In general, it is probably true to say that during this century competition has been keener and incentives have been fewer, and personal/

The distribution of deaths from
ischaemic heart disease between the
social classes (R. G. for Scotland).

PERCENTAGE
OF DEATHS
FROM
ISCHAEMIC
HEART
DISEASE
(1949 – 1953)

SOCIAL CLASSES

140.
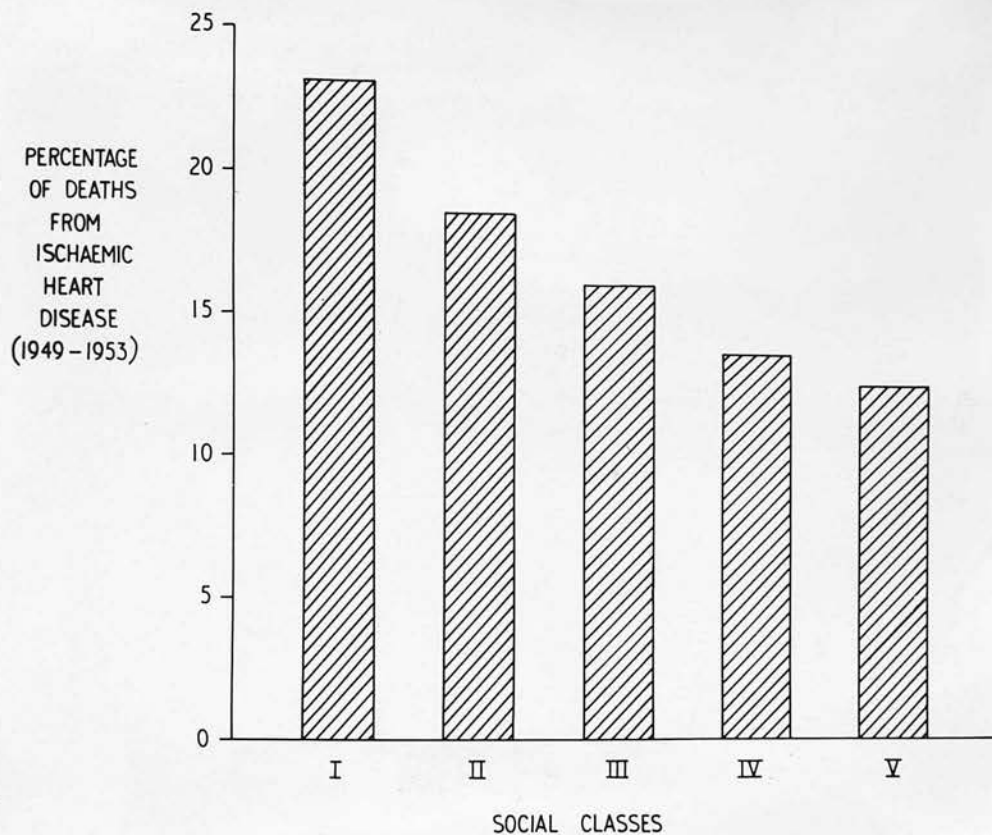
personal financial and emotional stability has
been less well balanced.   Exactly what relation
these changing environmental influences might
have to the rapid development of ischaemic heart
disease is at present obscure, but they should
not be overlooked as aetiological factors of
potential importance.

    c. <u>Endocrine imbalance</u>.

       It has already been postulated that an
endocrine imbalance may favour the development
of ischaemic heart disease and now some of the
possible disturbances of normal endocrine balance
will be considered.   The nature and precision
of each individual's endocrine balance may well
be determined constitutionally and by heredity,
and some individuals may be more susceptible
than others to extraneous influences which tend
to disturb this balance.   Any primary endocrine
dysfunction and particularly gross endocrine
diseases will disturb the physiological hormonal
balance.   Failure to withstand environmental
responsibilities might be transmitted to the
endocrine system by way of the cerebral cortex
and hypothalamus and result in disturbance of
normal endocrine balance.   Certainly acute
"stress"

"stress" can lead to profound endocrine dis-
turbances. For example, the normal pituitary-
adrenocortical balance can be upset and lead to
the various manifestations of the so-called "alarm
reaction", such as hypoglycaemia, gastrointestinal
erosions, haemoconcentration and eosinopenia;
the normal pituitary-thyroid balance can be
upset by acute psychic stress, and the rapid
development of thyrotoxicosis following a sudden
severe shock or acute anxiety is common and well
recognised; similarly, the normal pituitary-
gonadal balance can also be upset by acute
psychological duress, and the sudden cessation
of menstruation following extreme emotional
anxiety has often been described.   Thus, the
relationship between "stress" and disturbance
of endocrine gland function might be close, and
future investigations may indicate that the
aetiological role, which has been postulated in
this thesis, of an endocrine imbalance in the
production of ischaemic heart disease could be
partly dependent on increasing environmental
"stress" and decreasing ability to cope with it.

    d./

d. Ageing and local dynamic factors.

Ageing and local dynamic factors are
undoubtedly very important in the production of
ischaemic heart disease, but there is nothing to
suggest that they now have a greater influence
on the development of coronary atheroma than they
did thirty and forty years ago when ischaemic
heart disease was uncommon; moreover, there is
no satisfactory evidence that the incidence of
coronary atheroma has increased.   Advancing
age results in progressive loss of elasticity
leading to dilatation and stretching of the
arterial wall. This gradual deterioration with
age is something which elastic tissue has in
common with many similar colloidal substances,
and maybe the starting point of most of the
changes which occur with ageing (Ophüls, 1933).
From early youth there is a tendency to splitting
of the internal elastic lamina; at first this
is not very marked but it becomes more and more
complete with advancing age. Lansing (1955) has
shown that the aminoacid composition of young and
old arterial elastin is significantly different.

Subintimal/

Subintimal anoxaemia, fibrosis, lipid
deposition and calcification can occur in
arteries as a result of many local influences
including differences in intimal thickness,
elasticity tortuosity and intraluminal hydrostatic
pressure.

The possible aetiological relationship
of diet, environment, endocrine imbalance and
ageing is summarised in Fig. 37.
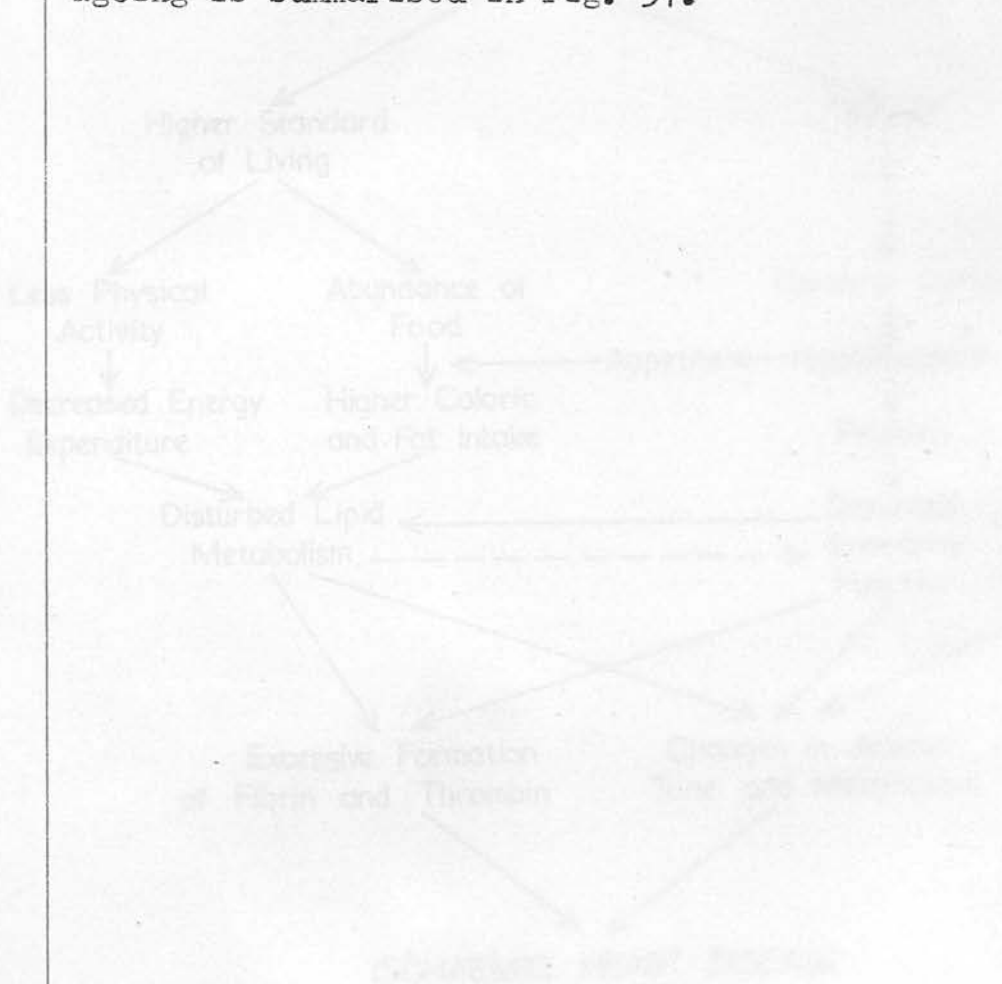
Fig. 37. A speculative scheme showing some of the possible relationships between various aetiological factors which favour the development of ischaemic heart disease.

Fig. 37. A speculative scheme showing some of the possible relationships between various aetiological factors which favour the development of ischaemic heart disease.
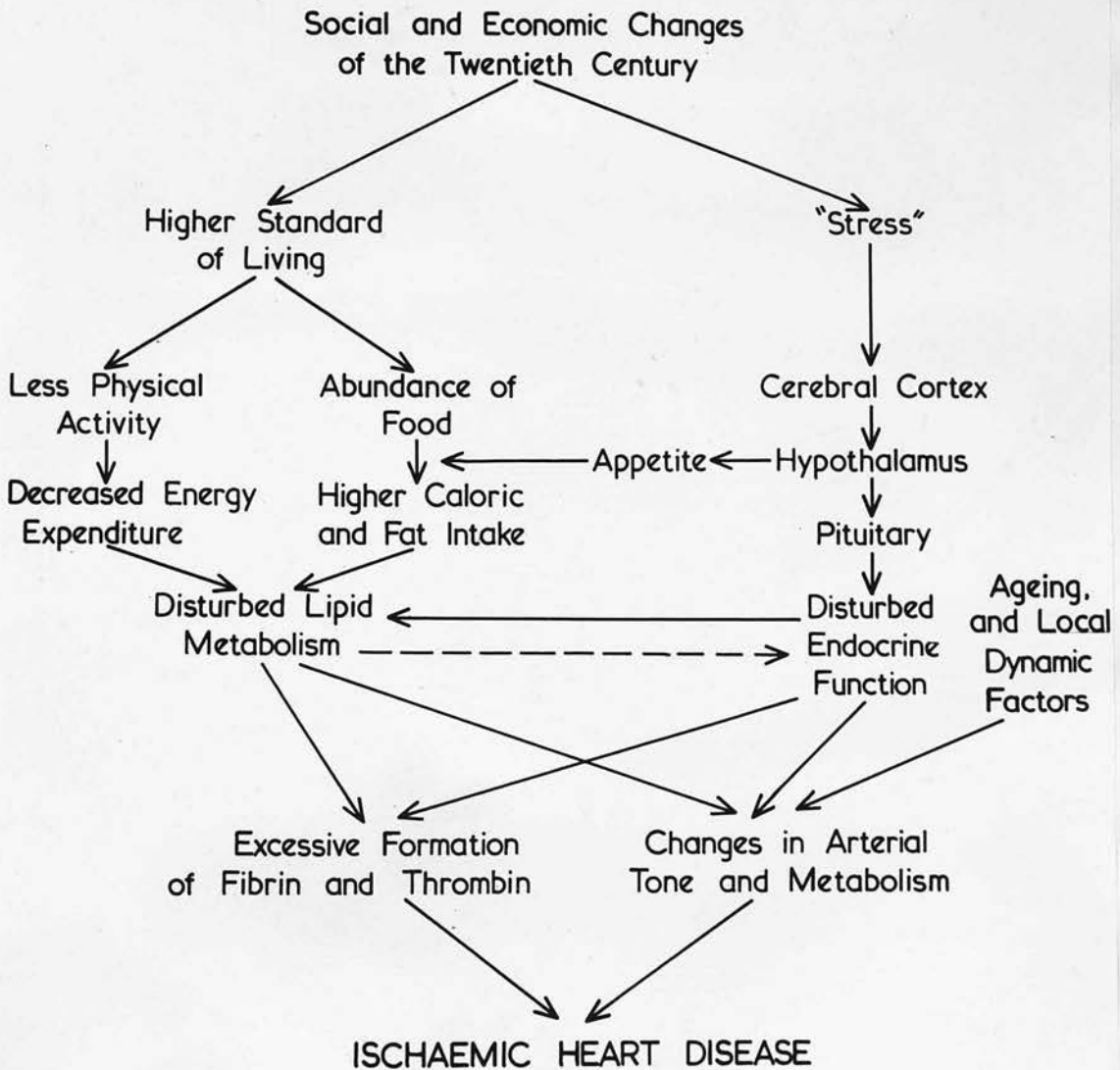
PART IV.

THERAPEUTIC IMPLICATIONS

The presence and significance of
abnormality of the circulating lipids and lipo-
proteins in patients with ischaemic heart disease
have already been described (Part II). The
blood coagulation and fibrinolytic systems and the
tonic and metabolic functions of the arterial
walls may also be disturbed in patients who are
prone to develop coronary atherosclerosis with
subsequent ischaemic heart disease. It may be
very important to prevent the deposition of
microscopic films of fibrin on the arterial
intima and to improve oxygenation to the arterial
wall, but at present so little is known about
these disturbances that no therapy can be
devised to correct them. Anticoagulants are
effective against the formation of frank intra-
vascular thrombosis, but even this action is not
entirely straightforward; for instance, anti-
coagulants which cause hypoprothrombinaemia do
not appear to retard clotting in vitro or in vivo
(Wessler, 1953) and may achieve reduction in
the incidence of thromboembolic episodes through
some other action in addition to their anticoag-
ulant properties (Gilchrist, 1952; Gilchrist &
Tulloch/

Tulloch, 1956).   It has already been indicated
that the abnormalities of blood coagulation and
the disturbance within the arterial wall may be
partially dependent on the presence of abnormal-
ities in the circulating lipids and lipoproteins,
and this section on therapy will, therefore,
be concerned solely with the desirability and
the possibilities of correction of these abnor-
malities.

A.   THE TREATMENT OF HYPERCHOLESTEROLAEMIA.

It would seem reasonable to assume that
reduction of the cholesterol in the plasma might
result in the passage of less cholesterol along
with the physiological diffusion of crystalloids
and colloids from the arterial lumen through the
arterial wall to the veins and lymphatics of the
adventitia.   In the experimental animal the
degree and extent of cholesterol deposition in
the arterial wall is directly proportional to
the degree of hypercholesterolaemia, thus re-
duction of hypercholesterolaemia is followed
experimentally by less cholesterol deposition.
Similarly, any improvement in the stability of
the colloidal dispersion of cholesterol in the
plasma probably also results in a decreased
tendency for cholesterol deposition./

deposition.   Such stability depends on the inter-
relationship of cholesterol with other lipids,
particularly the phospholipids, and with protein
(see Pages 48 - 50);   thus reduction of an
elevated C/P ratio and of an elevated $\beta:\alpha$
lipoprotein ratio might be regarded as desirable.
Lipaemia can accelerate coagulation and inhibit
fibrinolysis, and therefore correction of excessive
and frequent episodes of lipaemia might also be
beneficial.

1.   Diet.

Consideration of some of the causes which
could disturb the normal circulating lipids and
lipoproteins and contribute also to the increasing
incidence of ischaemic heart disease has implicated
dietary fat (see Page 136), and restriction of the
daily fat intake would therefore seem logical.   A
diet which is deficient only in cholesterol does
not produce any significant reduction of the elevated
circulating lipids and lipoproteins, but a diet low
in fat lowers these values.

a.   Low fat diet.

Twelve hypercholesterolaemic men have been
studied over 4 years: 8 had had a myocardial
infarct, 2 had angina and 2 were siblings of
hypercholesterolaemic/

hypercholesterolaemic patients with coronary

disease.   Two of the men, who had had a myo-

cardial infarct, died during the period of study

and the results reported in Fig. 38 have been

obtained from the remaining 10 men.   These subjects

were selected as intelligent and cooperative men

whose sedentary occupations would not demand a

high caloric intake.   It was appreciated from the

start that a low fat diet would be unpalatable,

monotonous and difficult to observe strictly,

and so a small group of cooperative men, mostly

in clerical occupations, were selected in

preference to a larger and more varied group.

The initial daily regime consisted of 1200

calories with 20 g. of fat, or 15% of the total

calories from fat.   After 6 months this was

increased in 7 men to 1600 calories with 26 g.

of fat, which also represents 15% of the total

calories from fat.   One of the remaining 3 men

received 1400 calories and 24 g. of fat, and the

other 2 men received 1800 calories with 30 g.

of fat.   Thus, although there was some variation

in the caloric intake, the contribution of fat

never exceeded 15% of the total.   These men have

been/

Fig. 38. The mean changes in the circulating lipids following the ingestion of a low fat diet by 10 hypercholesterolaemic men with ischaemic heart disease. The average intake was approximately 1400 calories and less than 25 g. of fat; during the last year the diets may have been rather more liberal in calories but have never exceeded 2000 and more than 15% from fat.

< 25g FAT & 1400 CALORIE DIET

PLASMA CHOLESTEROL mg %
400
300
200
K

PLASMA C/P RATIO
1·20
1·10
1·00
0·90
0·80

BODY WEIGHT Kg
52
50
48

-12 -6 -0 3 6 9 12 18 24 30 36 42 48
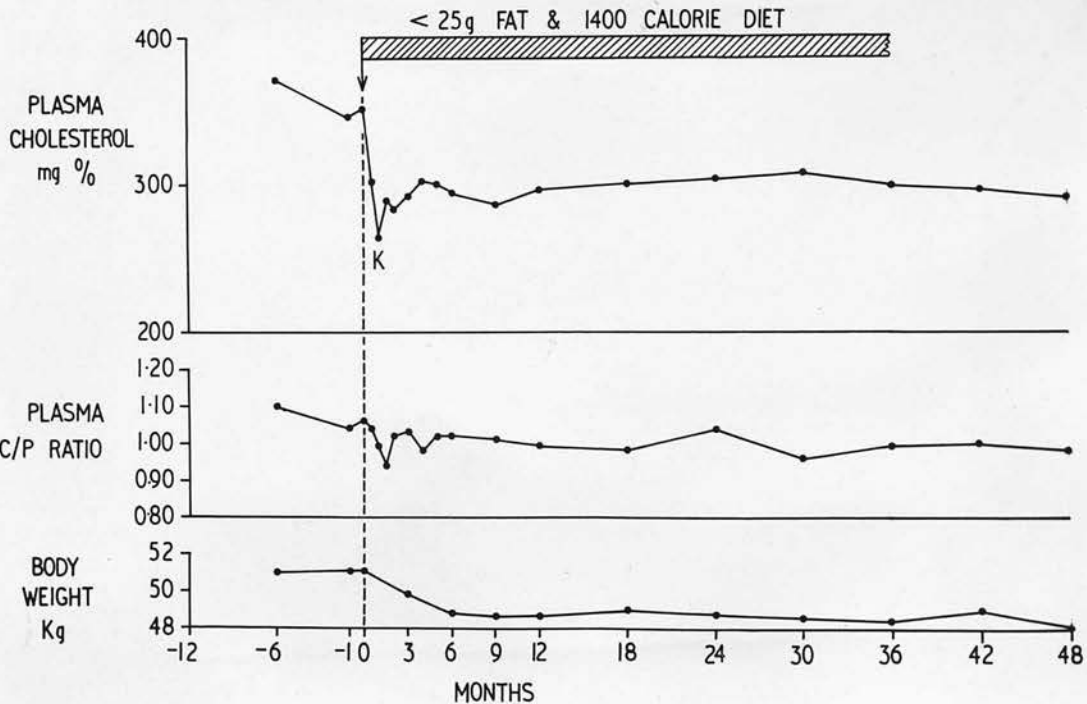
MONTHS

been followed at 3 monthly intervals and it is
believed that, while there may have been occasion-
al lapses, they have observed their diets strictly.
From Fig. 38 it can be seen that there was an
initial rapid fall in plasma cholesterol and at
the nadir this was associated with ketonuria,
presumably indicating some endogenous mobilisation
of fat reserves.   This harsh dietary regime
eventually achieved no more than a 15% reduction
in plasma cholesterol.   Even if more complete
removal of fat from the diet could be tolerated
for any length of time, no very striking reduction
of plasma cholesterol would be expected as
cholesterol is synthesised in the liver from
active acetate derived from all three dietary
elements, protein, carbohydrate and fat (see
Page 51).   A totally fat-free rice-fruit diet
will, however, achieve marked reduction of plasma
cholesterol (Kempner, 1948).

It is concluded that severe restriction
of dietary fat is unpleasant and often intolerable
and does not achieve great reduction of the cir-
culating lipids and lipoproteins, although
dramatic effects can sometimes be achieved (Fig.
39).   In/

Fig. 39.   The changes in the circulating lipids
            and lipoproteins following the ingestion
            of a low fat diet by a patient with
            "idiopathic" hypercholesterolaemia and
            hyperlipaemia.
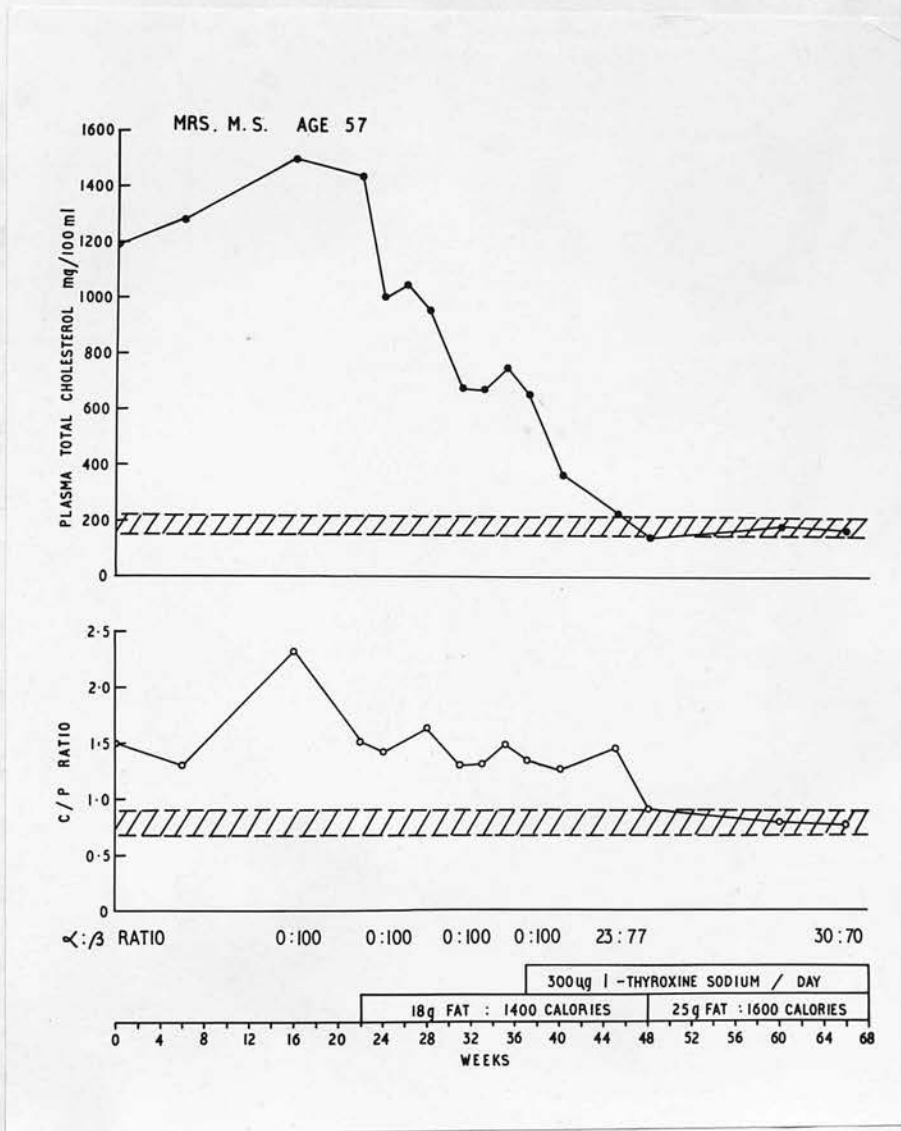
In 1955 the average man in Great Britain received 40% of his calories from fat, and it is possible that less severe restriction of fat to between 25% and 30% of the caloric intake might be more practical and might achieve slight reduction of the circulating lipids and lipoproteins.

b. <u>Vegetable oil diet</u>.

Evidence has been accumulating recently that an increase in the intake of vegetable fat causes depression of the circulating cholesterol, C/P ratio and β-lipoprotein cholesterol (Kinsell <u>et al</u>, 1952, 1953; Beveridge <u>et al</u>, 1955; Malmros and Wigand, 1955; Bronte-Stewart <u>et al</u>, 1956). It appears that this effect of vegetable and fish oils is roughly but directly proportional to their lack of saturation and is probably related to their essential fatty acid content. Whereas this effect of vegetable oils may lead to a practical therapeutic regime, at present an elaborate dietetic organisation is necessary to disguise the large quantity (200 g) of oily emulsion which has to be swallowed each day. So far the author has not administered any vegetable oil to hypercholesterolaemic subjects but the/

the administration of sunflower seed oil, which
acted as a severe stomach and bowel irritant, to
healthy young men did not induce any significant
depression of cholesterol below normal levels.

    c.   <u>Plant Sterols</u>.

    Sitosterol, a stereoisomer of cholesterol,
reduces the absorption of cholesterol from the
diet and the reabsorption of cholesterol excreted
in the bile;   this effect may be achieved by
competition for esterification which is necessary
for the absorption of most intestinal cholesterol.
The administration of sitosterol is followed by
some reduction in circulating cholesterol in most
hypercholesterolaemic patients (Pollak, 1953).
The daily oral administration of 10 g. of β -
sitosterol for 14 days to 4 hypercholesterolaemic
men with coronary disease produced depression
of the level of the circulating cholesterol by 10%
and of the β-lipoprotein cholesterol by 8%.   Sito-
sterol is a tasteless bran-like powder, which has
to be sprinkled over all food making it dry and un-
palatable.   Plant sterols do not impair the ab-
sorption of carbohydrate and protein, and the endo-
genous synthesis of cholesterol is also unlikely
to be affected.   It is improbable, therefore,
that/

that plant sterols will have any useful thera-
peutic application.

    d.   Lipotrophic factors.

       Lipotrophic factors, such as choline and
methionine, prevent fatty livers and are said to
promote phospholipid synthesis thereby improving
the transport and stability of cholesterol.
Despite individual reports to the contrary, there
has been no satisfactory evidence that they
influence the circulating cholesterol in man
(Davidson, 1951).   The daily oral administration
of 3 g. of choline chloride for 2 weeks to 6
hypercholesterolaemic patients with ischaemic heart
disease did not result in any significant change
in the plasma cholesterol or C/P ratio.

    2.   Hormones.

       Three groups of hormones have been shown
to lower hypercholesterolaemia; they are
pituitary-adrenocortical hormones (ACTH and
cortisone), thyroid hormones and the oestrogenic
sex hormones.   Since ACTH and cortisone have
fundamental and widespread metabolic actions in
addition to their action on circulating cholesterol,
they are obviously precluded from any attempt to
achieve/

achieve permanent reduction of hypercholesterol-
aemia.   Thyroid hormones and the oestrogenic
sex hormones have not been administered for the
control of hypercholesterolaemia in many centres
outside Edinburgh, and thus they will be considered
in some detail.

   a.   Thyroid hormones.

        The plasma cholesterol, C/P ratio and
 β -lipoprotein cholesterol are all significantly
depressed during administration of l -thyroxine
sodium, l -triiodothyronine and triiodothyroacetic
acid (triac) to euthyroid hypercholesterolaemic
men with coronary disease;   this influence of
these thyroid derivatives has already been
considered in detail (see Pages 115 - 119).
Unfortunately thyroxine itself and these analogues
of thyroxine increase the basal metabolic rate
and thus increase the work and oxygen consumption
of the myocardium. When the blood supply is
impaired by coronary atherosclerosis, any increase
in the demands by the myocardium might be expected
to result in temporary ischaemia and thus   several
of the men developed angina.   This aspect of
therapy with thyroxine derivative will now be
discussed.

   i.   Thyroxine./

154.

i.   Thyroxine.

It has already been reported that 6
euthyroid hypercholesterolaemic men with coronary
disease received 600 μg. of l-thyroxine sodium
daily for 2 weeks with depression of the circul-
ating lipids and lipoproteins and concomitant
elevation of the basal metabolic rate by a mean
of +13% (see Fig. 24).   Three of these men ex-
perienced appreciable reduction of their exercise
tolerance due to the development of effort angina,
which regressed completely a few weeks after
thyroxine had been discontinued.

ii.   Triiodothyronine

Three euthyroid hypercholesterolaemic
men with coronary disease received up to 100 μg.
of l-triiodothyronine daily for 6 weeks with
depression of the circulating lipids and lipo-
proteins and concomitant elevation of the basal
metabolic rate by a mean of +11% (see Fig. 25).
Two of these 3 men experienced significant in-
crease in the frequency of angina with subsequent
regression when they received identical placebo
tablets.

A further 3 euthyroid hypercholesterol-
aemic men with coronary disease received up to
60 μg./

60 μg. of l-triiodothyronine daily over 7 weeks with depression of the circulating lipids and lipoproteins without any increase in basal metabolic rate (see Fig. 40). However, one man who had previously had an excellent exercise tolerance developed effort angina for the first time in 3 years; he had no rise in basal metabolic rate.

iii. Triiodothyroacetic acid.

Six euthyroid hypercholesterolaemic men with coronary disease received from 0.5 mg. to 4 mg. of triac daily over 95 days with depression of the circulating lipids and lipoproteins above a dose of 3mg. (see Fig. 26). In two men there was elevation of the basal metabolic rate by 20% and 13% respectively. One of these two men and one of the remaining four men, in whom there was no elevation of basal metabolic rate, complained of marked reduction of exercise tolerance due to effort angina. When triac was withdrawn and placebo tablets started both these men experienced rapid regression of their symptoms and soon returned to their normal exercise tolerance.

A further 6 euthyroid hypercholesterolaemic men with coronary disease received from 3.0/

Fig. 40. The changes in the circulating lipids and lipoproteins, and in the weight and basal metabolic rate, during the oral administration of 40 and 60 μg. of *l* - triiodothyronine daily to 3 euthyroid hypercholesterolaemic men with ischaemic heart disease.
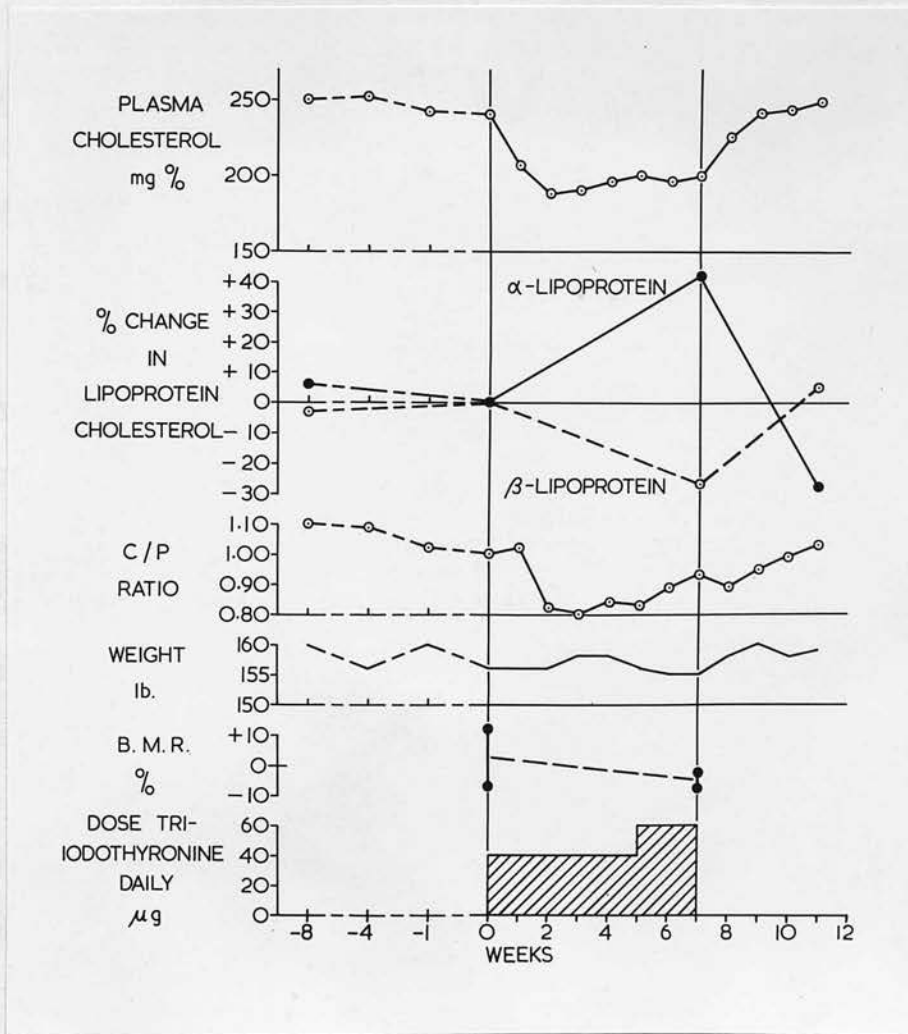
3.0 to 5. 0 mg. of triac daily over 12 weeks with
depression of the plasma cholesterol, C/P ratio
and β-lipoprotein cholesterol at the 3 and 4 mg.
dose levels (see Fig. 41). Despite the continued
administration of 4 mg. of triac daily these
values rose to the control levels, but there was
a further slight fall when 5 mg. was administered
daily although this was much less impressive than
the original fall. Withdrawal of triac resulted
in a rebound rise in the lipid and lipoproteins.
The basal metabolic rate of these men was not
significant altered, but one of these 6 men
developed more angina and exertional breathlessness
with reduction of exercise tolerance (Oliver & Boyd, 1957)

In summary, 24 euthyroid hypercholesterol-
aemic men with coronary disease, received thyroxine
or one of its analogues. The circulating lipids
and lipoproteins of them all were significantly
lowered but 9 experienced definite reduction of
exercise tolerance. This is readily understand-
able in 6 men, whose basal metabolic rates were
elevated, but less so in the remaining 3 men, in
whom there was no elevation of basal metabolic
rate. It is interesting, and possibly important
therapeutically, that the circulating lipids and
lipoproteins/

Fig. 41. The changes in the circulating lipids
and lipoproteins, and in the basal meta-
bolic rate and weight, during the oral
administration of increasing large doses
of triiodothyroacetic acid to 6 hyper-
cholesterolaemic euthyroid men with
ischaemic heart disease.
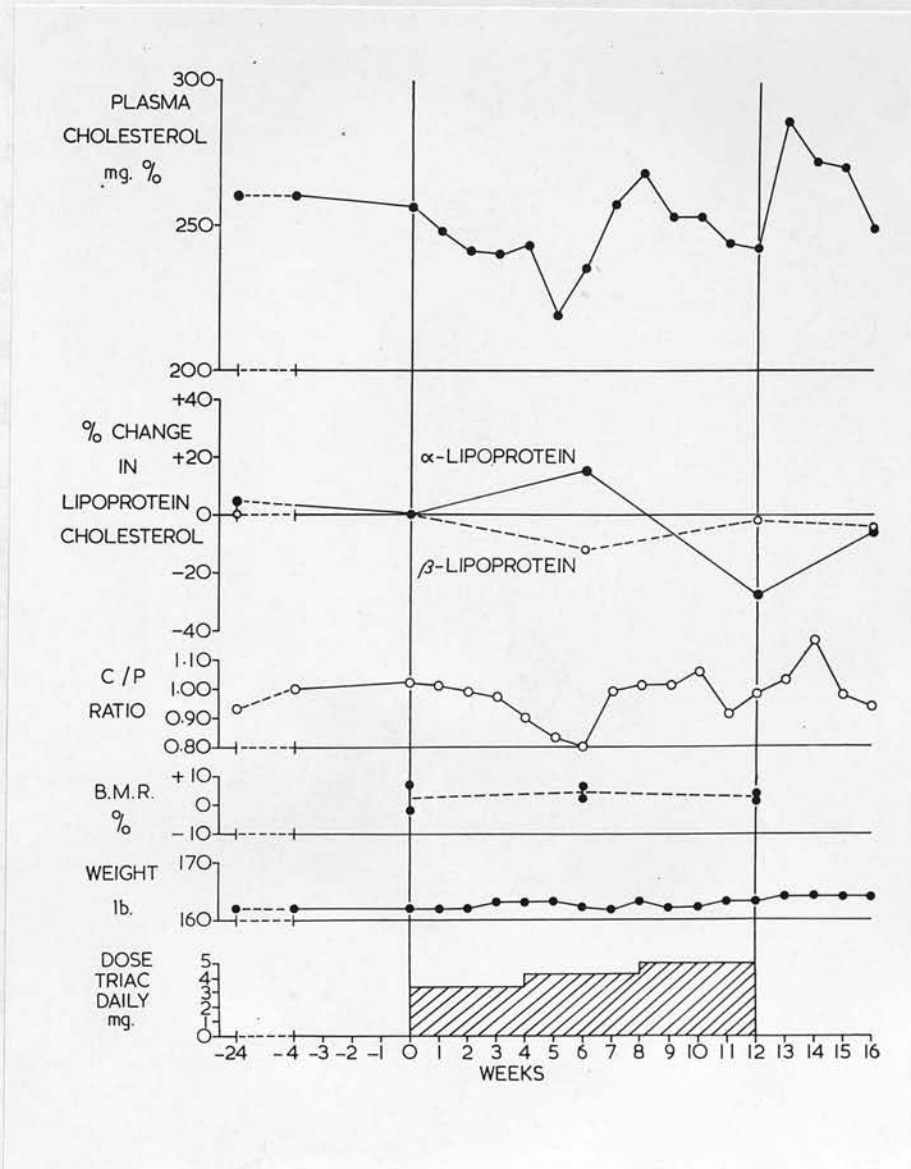
lipoproteins can be lowered by small doses of triiodothyronine and triiodothyroacetic acid without elevation of the basal metabolic rate (Figs. 26, 40 and 41).   It has been demonstrated in rats that triac increases the oxygen uptake of heart tissue to a greater extent than that of liver, skeletal muscle and kidney (Barker & Lewis, 1956; Boyd & Oliver, 1957). The measurement of the basal metabolic rate cannot reflect the relative oxygen requirements of all the body tissues and hence the administration of a substance, which produces a biological response (plasma cholesterol depression) without influencing the basal metabolic rate, may well alter the oxygen requirement of certain specific tissues. It is possible, therefore, that the dissociation of the action of triac on the circulating lipids and lipoproteins from any effect on the basal metabolic rate is more apparent than real, and may depend partly on inadequacy of the basal metabolic rate measurement to reflect a selective increase in the oxygen requirements of a comparatively small tissue.   Triac not only induced angina but also failed to maintain depression of the/

the circulating lipids and lipoproteins despite
increasing doses (Fig. 41).   It is concluded
that it is unlikely that triiodothyronine or
triac, despite their insignificant action on the
basal metabolic rate, will prove suitable for the
long-term control of hypercholesterolaemia in
patients with clinical coronary disease.   Future
investigations should be directed towards the
discovery of a thyroxine analogue which will not
only depress the circulating lipids without
altering the overall metabolic rate but also will
not cause angina or any selective increase in
oxygen uptake by heart tissue.

    b.   Oestrogenic sex hormones.

        It has already been shown that the plasma
cholesterol, the C/P ratio and the β-lipoprotein
cholesterol are all significantly and consistently
depressed during the administration of any
oestrogen (see Pages 119 - 122).   Ethinyl oestra-
diol produced the most significant depression of
the circulating lipids and lipoproteins.   The vast
majority of the 125 men, who have received oestro-
gens, have experienced gynaecomastia in varying
degree, and in 2 of these men it was severe enough
to cause withdrawal of the oestrogen preparation.
Depression/

159.

Depression of libido and loss of libido in older

men occurred in 42% and nausea occurred

occasionally.   The frequency of these side

effects of oestrogen therapy are listed in

Table XXV.

Table XXV.   The side effects of oestrogen
              therapy in 125 hypercholesterolaemic
              men with coronary disease.

Side effects	Percentage of men
Gynaecomastia	98%
Depression or loss of libido	42%
Nausea	12%
Penile irritation	3%
Testicular pain	3%

Despite the frequency of gynaecomastia and

depression of libido it is remarkable how well
two
these/feminising effects have been tolerated.   An

attempt was made to minimise these feminising

effects by the combined administration of an

androgen or a progestin with ethinyl oestradiol.

    i.   Androgen and oestrogen.

    (1) Methyl testosterone + ethinyl oestradiol:

        12 hypercholesterolaemic men with proven

coronary disease were studied over 68 weeks.

5 mg. of methyl testosterone were administered

sublingually/

160.

sublingually each day for 4 weeks, and 10 mg for
a further 4 weeks. During these 8 weeks the men
also received inert tablets similar in size and
shape to ethinyl oestradiol. At the end of 8 weeks
tablets containing 400 μg. of ethinyl oestradiol
were substituted for the inert tablets, and for
the next 14 weeks the men received increasing
doses of ethinyl oestradiol and 10 mg of methyl
testosterone daily; after the first 2 weeks
600 μg. of ethinyl oestradiol were administered
each day and after 2 more weeks 800 μg. then after
2 further weeks 1 mg. of ethinyl oestradiol was
administered daily.   At the end of 14 weeks
combined therapy inert sublingual tablets identical
to the androgen were substituted, and the men
received 1 mg. of ethinyl oestradiol daily for 4
more weeks when idential inert tablets were again
substituted for the oestrogen; the men then
received two groups of inert tablets for a further
12 weeks.

        The results of this study are shown in
Fig. 42.   The plasma cholesterol did not undergo
any appreciable fall until a dose of 1 mg. of
ethinyl oestradiol was reached, but when methyl
testosterone/

Fig. 42. The changes in the circulating lipids
during the combined oral administration
of methyl testosterone and ethinyl oest-
radiol to 12 hypercholesterolaemic men
with ischaemic heart disease.
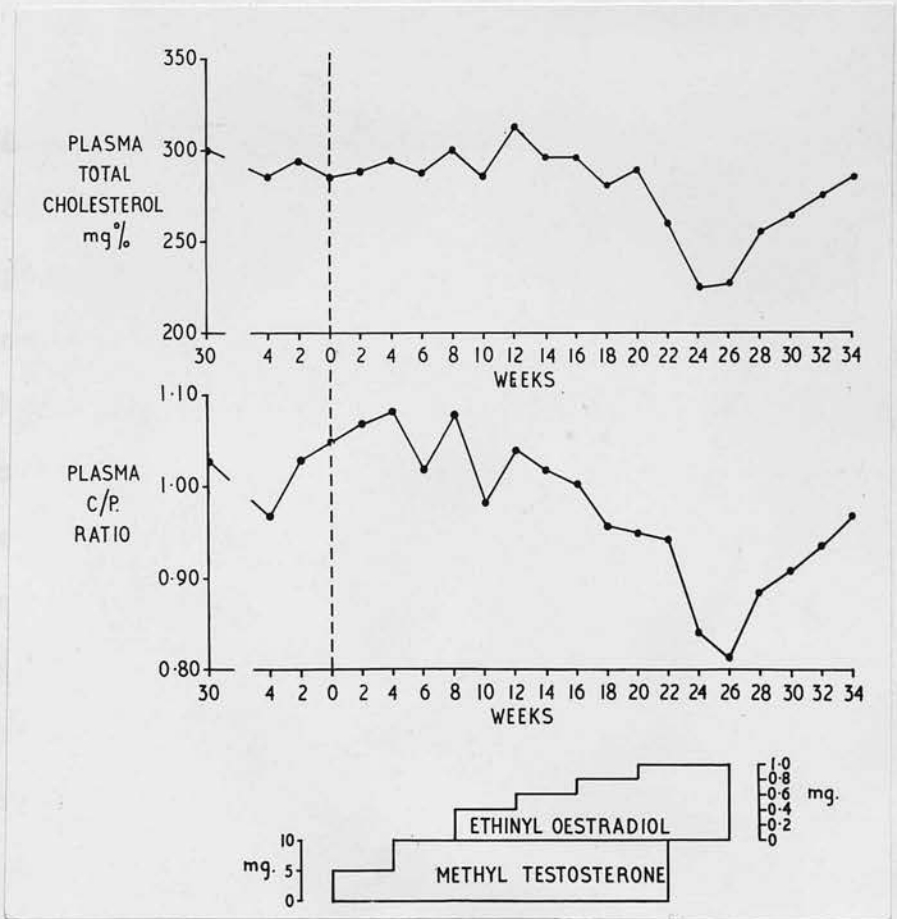
testosterone was withdrawn, the plasma cholesterol
fell by 21%.   The C/P ratio did not undergo
any appreciable fall until 800 μg. of ethinyl
oestradiol was being received daily but when
methyl testosterone was withdrawn, the C/P ratio
fell by 23%.

(2) Ethinyl oestradiol + methyl testosterone.

A further 12 hypercholesterolaemic men
with proven coronary disease were studied for
30 weeks.   200 μg. of ethinyl oestradiol were
administered daily for 2 weeks, and thereafter
up to the end of the 10th week the daily dose
was increased by 200 μg. every 2 weeks.   During
these 10 weeks the men also receive inert subling-
ual tablets similar in size, shape and taste to
methyl testosterone sublingual tablets.   At the
end of these 10 weeks sublingual tablets con-
taining 20 mg. of methyl testosterone were
substituted for the inert tablets, and for the
next 4 weeks the men received the androgen and
1 mg. of ethinyl oestradiol daily.   After 4 weeks
combined therapy inert tablets identical to the
oestrogen were substituted, and the men received
20 mg. of methyl testosterone daily for 4 more
weeks/

weeks when inert sublingual tablets were again
substituted for the androgen; the men then
received two groups of inert tablets for a further
4 weeks.

The results of this study are shown in
Fig. 43. The plasma cholesterol fell by 25%
after 10 weeks of ethinyl oestradiol. When
methyl testosterone was introduced, the plasma
cholesterol rose by 35%, and when ethinyl oestra-
diol was withdrawn, the plasma cholesterol rose
a further 12%. The C/P ratio fell by 25% after
10 weeks of ethinyl oestradiol. When methyl
testosterone was introduced, the C/P ratio rose
by 20%, and when ethinyl oestradiol was withdrawn,
the C/P ratio rose a further 19%.

The administration of methyl testosterone
in these two studies not only failed to modify
or prevent any of the side effects of ethinyl
oestradiol, but resulted in partial inhibition
of the depressant action of ethinyl oestradiol
on the circulating lipids.

ii. Progestin + oestrogen

Ethinyl testosterone + ethinyl oestradiol

12 hypercholesterolaemic men with proven
coronary/

Fig. 43. The changes in the circulating lipids during the combined oral administration of ethinyl oestradiol and methyl testosterone to 12 hypercholesterolaemic men with ischaemic heart disease.
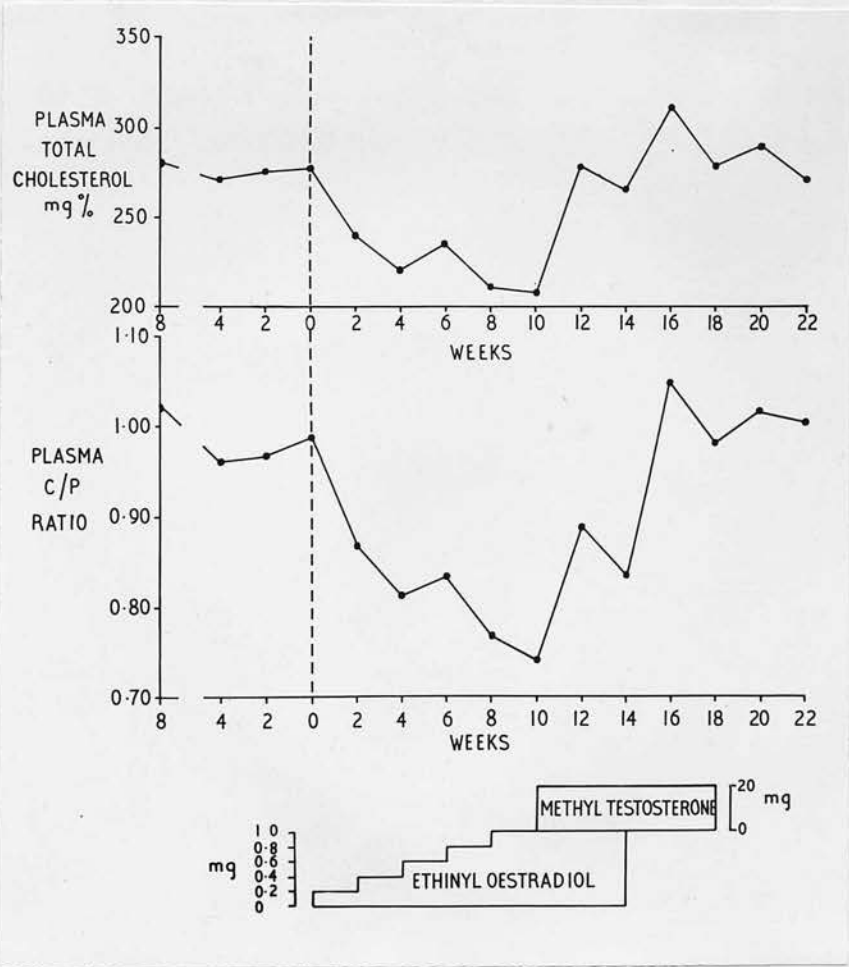
coronary disease were studied for 26 weeks.
20 mg. of ethinyl testosterone (anhydro-
hydroxyprogesterone) were administered sublingually
each day for 2 weeks; thereafter 40 mg. were
administered daily for 2 weeks and then 60 mg.
were administered daily for the next 2 weeks.
During these 6 weeks the men also received inert
tablets similar in size and shape to ethinyl
oestradiol. At the end of 2 weeks, tablets
containing ethinyl oestradiol 500 μg. were
substituted for the inert tablets, and for the
next 4 weeks the men received the oestrogen and
60 mg. of the progesterone. analogue daily;
after 2 weeks 1 mg. of ethinyl oestradiol was
administered daily.   At the end of 4 weeks of
combined therapy inert sublingual tablets identical
to the progesterone analogue were substituted, and
the men received 1 mg. of ethinyl oestradiol daily
for 2 more weeks when identical inert tablets
were again substituted for the oestrogen; the
men then received two groups of inert tablets
for a further 8 weeks.

    The results of this study are shown in
Fig. 44/

Fig. 44.　The changes in the circulating lipids
during the combined oral administration
of anhydrohydroxyprogesterone (ethinyl
testosterone) and ethinyl oestradiol
to 12 hypercholesterolaemic men with
ischaemic heart disease.
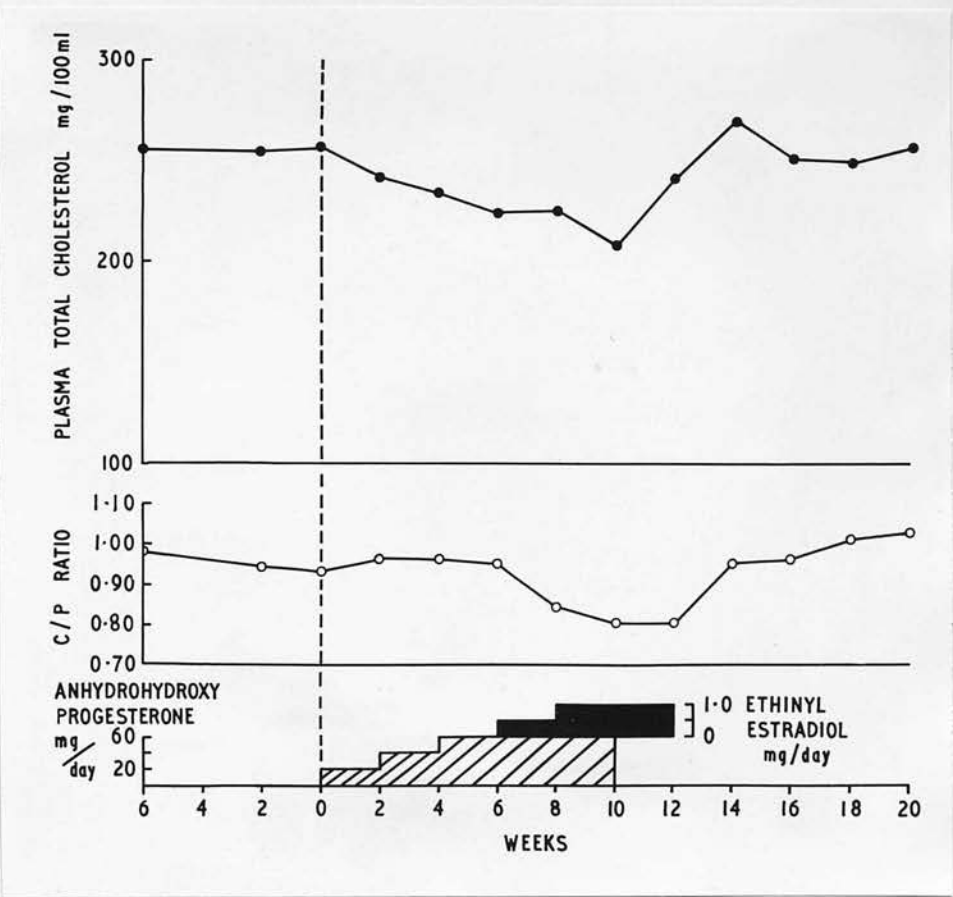
Fig. 44.   The plasma cholesterol fell by 12%
after 6 weeks of ethinyl testosterone.   It fell
by a further 10% when 1 mg. of ethinyl oestradiol
was administered at the same time.   There was
a rise in plasma cholesterol by 18% when ethinyl
testosterone was withdrawn even although the men
were still receiving 1 mg. of ethinyl oestradiol
daily, and there was a further rise on withdrawal
of ethinyl oestradiol.   The C/P ratio did not
alter significantly during the 6 weeks when the
men received ethinyl testosterone, but it fell
by 13% when ethinyl oestradiol was introduced.
The C/P ratio subsequently rose by 29% following
withdrawal of ethinyl oestradiol.   This combined
administration of a progestin with ethinyl
oestradiol failed to ameliorate any of the
feminising side effects of oestrogen therapy.

In summary, there is no doubt that the
oestrogenic sex hormones lower the abnormal
circulating lipids and lipoprotein patterns present
in clinical coronary disease, but their feminising
actions are likely to preclude them from any
attempt at long-term control of hypercholesterol-
aemic patients with clinical coronary disease.
Nevertheless,/

Nevertheless, a long-term study of the effect of oestrogens on the circulating lipids and on the rates of morbidity and mortality from ischaemic heart disease is being conducted. The subjects are 100 men with proven myocardial infarction; these men were discharged consecutively from Ward 22 of the Royal Infirmary and ever since alternate cases have received 200 μg. of ethinyl oestradiol daily or identical inert tablets. The men attended as outpatients every 3 months during the first year after discharge from the ward and have attended 6 monthly thereafter; the clinical features, blood pressure, weight and a blood specimen for lipid analysis have been assessed at each visit, and an electro-cardiogram has been recorded at every second visit. The majority have been followed for $1\frac{1}{2}$ years and 25 for more than 2 years. The effect of ethinyl oestradiol on the circulating lipids is shown in Fig. 45. In contrast to triiodothyro-acetic acid, ethinyl oestradiol maintains depression of the plasma cholesterol and C/P ratio over long periods; the greater effect on the C/P ratio emphasises that oestrogens/

Fig. 45.   The changes in the circulating lipids
            during the oral administration of 200
            μg. of ethinyl oestradiol daily on a
            long-term basis.   The number of cases
            in the control group and oestrogen
            treated group are also shown.
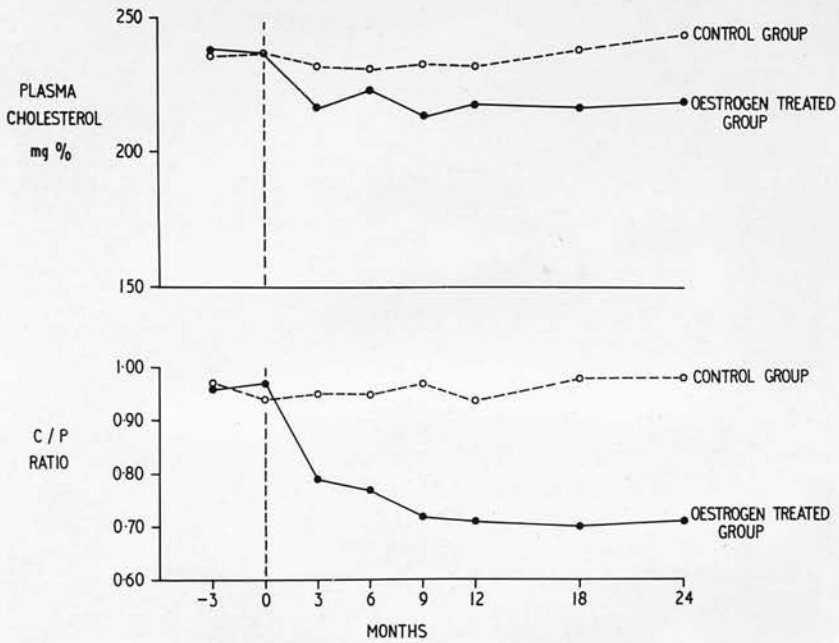
oestrogens cause elevation of the phospholipids
as well as depression of cholesterol.   Up to
the present there have been 6 deaths in the oes-
trogen treated group and 4 deaths in the control
group;   it is unlikely that this study will give
any reliable or valuable information concerning
the influence of lowered circulating lipids on
morbidity and mortality until it has been pursued
for 5 years.

3.   Miscellaneous.

Several miscellaneous substances such
as large doses of heparin (Basu & Stewart, 1952),
nicotinic acid (Altschul & Herman, 1955),
chelating agents (Perry & Schroeder, 1955), and
phenyl ethyl acetic acid (Mathivat & Cottet,
1953) have been reported to lower plasma cholest-
erol.

Phenyl ethyl acetic acid, or "hyposterol",
has been administered at three dose levels to 18
hypercholesterolaemic men with proven coronary
disease.   No significant change was produced in
6 men during the daily oral administration of
1.2 g.. (optimum dose according to the original
investigators) for 12 weeks or in a further 6 men
during the daily oral administration of 2.7g.
for/

Table XXVI. The effect of phenyl ethyl acetic acid on the circulating
lipids and lipoproteins in hypercholesterolaemic men with
ischaemic heart disease.

	Number of subjects	Plasma cholesterol	C/P ratio	β-lipoprotein cholesterol	α-lipoprotein cholesterol
1.2 g./ day for 12 weeks	6	+8%	+13%	-	-
2.7 g./ day for 4 weeks	6	+11%	+4%	+3%	-7%
5.0 g./ day for 2 weeks	6	-17%	+7%	No significant change	

for 4 weeks.   However, the daily oral
administration of 5g. to a further 6 men resulted
in slight depression of the mean plasma cholest-
erol by 17% (Table XXVI).   Although apparently
not toxic after a few weeks, phenyl ethyl acetic
acid has not yet been administered over long
periods.   At present it is not regarded as a
potent cholesterolytic agent.

B. CONCLUSION.

At present there is no really effective
acceptable method of lowering hypercholesterol-
aemia.   Restrictions or alterations of the diet
are unpleasant and monotonous, and do not achieve
any very marked or consistent depression of
hypercholesterolaemia.   Thyroid analogues and
oestrogens achieve the most marked and consistent
depression of the elevated circulating lipids
and lipoproteins but the former increase myocardial
metabolism even in the absence of any effect on
the basal metabolic rate, and the latter induce
feminisation to a greater or lesser degree in
every subject.   It is possible that intensive
investigation of new thyroid analogues will lead
to a derivative which will have little or no
effect/

effect on metabolism but continue to depress
cholesterol.　It is also possible that a
"non-feminising oestrogen" will be developed,
although such a preparation may also lose its
cholesterolytic action.

It is on a study of prognosis, and not
on a study of reduction of circulating cholest-
erol that the efficiency of any particular regime
must be judged.　It is fundamental to determine
whether correction of the abnormal circulating
lipids and lipoproteins is associated with an
improvement in morbidity and mortality, and
particularly with inhibition or even regression
of the atherosclerotic lesions.　This is
obviously difficult to investigate in man, althou-
gh some indication that the atherosclerotic pro-
cess might be halted is obtained from two recent
studies.　Autopsies of middle-aged men who died
in Russian prisoner-of-war camps after months
or years of extreme hunger, and autopsies of men
who died during the hunger blockade of
Leningrad in 1942 were remarkable for the fre-
quent absence of any atherosclerosis in the
coronary arteries and for the very rare occurrence
of/

169.

of significant atherosclerotic lesions; moreover,
myocardial infarcts were practically never found
(Pierach, 1955). Similarly, autopsies of men,
who died from carcinoma of the prostate after
receiving large doses of oestrogens, showed less
coronary atherosclerosis than a comparable control
group (Rivin & Dimitroff, 1953). It is encour-
aging to think that the atherosclerotic process
might have been retarded in the subjects of
these studies. Yet once atherosclerotic lesions
have become extensive and severe enough to
produce clinical features of ischaemic heart
disease, inhibition of the atherosclerotic pro-
cess may not necessarily prolong life; when an
efficient and acceptable therapy can be proved
to retard the atherosclerotic process in the
human, it should probably be offered primarily
to the younger and apparently healthy members of
the community. Any such prophylactic regime
will depend on the perfection of reliable tests
so that those individuals who are likely to
develop ischaemic heart disease can be selected
with certainty from the healthy population.

The/

SUMMARY

The early history, the nature and the
incidence of coronary atherosclerosis and
ischaemic heart disease have been reviewed.
Evidence has been presented to suggest that there
are aetiological factors which favour the develop-
ment of ischaemic heart disease without influencing
the incidence of coronary atherosclerosis.

The close relationship of cholesterol
metabolism to coronary atherosclerosis and to
ischaemic heart disease has been emphasised.

The circulating lipids and lipoproteins
have been studied in health in relation to age
and sex.   They were abnormal in the majority
of patients with ischaemic heart disease,
particularly in those under the age of 50.
Analysis of the circulating lipids and lipoproteins
could aid the solution of an equivocal diagnosis
in young subjects suspected of having ischaemic
heart disease.

The/

171.

The effects of endogenous hormones and the action of administered hormones on the circulating lipids and lipoproteins have been described in detail.   Hormones can also influence the fluid state of the blood and the tonicity and metabolism of arteries.   It has been postulated that the homeostasis of cholesterol metabolism, of the fluid state of the blood and of arterial metabolism could be disturbed by alteration of the physiological endocrine balance.

The thesis has been proposed that an endocrine imbalance could contribute to the development of coronary atherosclerosis and of ischaemic heart disease.   The relationship of this thesis to existing theories of the aetiology of ischaemic heart disease has been considered in detail.

Finally, some therapeutic implications of these metabolic and endocrine aspects of coronary disease have been considered.

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investigations.   I am also greatly indebted to
Dr. George Boyd, who has been a close collabor-
ator in a number of these investigations; he
has conducted some comparable studies in animals,
from which much useful information has been
gained, and his expert knowledge of sterol bio-
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173.

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