URINARY OESTROGEN LEVELS IN MYOCARDIAL INFARCTION

I. BERSOHN, B.Sc., M.B., B.Ch. (RAND) and P. J. OELOFSE, M.Sc. (POTCH.)

Ernest Oppenheimer Heart Research Unit, South African Institute for Medical Research, Johannesburg

It is well known and generally recognized that there is a marked sex difference in the incidence of myocardial infarction and that coronary thrombosis occurs less frequently in premenopausal women than it does in men of comparable age. In the Union of South Africa between 1926 and 1954 the toll of coronary disease increased from 0·9% to 19·6% for males and from 0·53% to 11·6% for females. In 1954 the mortality rate from coronary artery disease was 177·0 and 74·3 per 100,000 for males and females respectively.[1] Smit has also shown that in the younger age groups relatively more males than females died of this disease, and that the difference in mortality rate progressively decreased with the increase in the age of the group.

An analysis of 1,000 consecutive cases attending the Edinburgh Royal Infirmary, all of whom had evidence of myocardial infarction or ischaemia, indicated an incidence 15 times higher in men than in women under the age of 40; an incidence 7 times higher in men under the age of 50 and only a 2 : 1 ratio of men to women under the age of 60. Oliver and Boyd, in view of the above findings, suggested that since the incidence of coronary artery disease increases after natural or artificial menopause, the development of the disease may be accelerated by changes in hormonal balance.[2]

Hueper[3] states that atherosclerosis (as exemplified by coronary sclerosis) occurs much more commonly in men than in women, and that the recent increase in the frequency of coronary artery disease in the USA affected men more than women.

In England and Wales in 1954, deaths attributed to coronary artery disease were 41,688 males and 23,983 females. The disparity in sex distribution was most evident in the younger age groups where the male female ratio was 5-6 : 1 whilst in in the older age groups 75 years and over, there was a ratio of less than 2 : 1.[4]

Lee and Thomas[5] have shown that in the older age groups the incidence of acute myocardial infarction in females is proportionately greater than in males. The incidence in women rose steadily with age, but was stationary in males from 60 to 80 years of age, when the incidence decreased. These findings were consistent with the theory that female sex hormones inhibit the development of atherosclerosis since these hormones decrease progressively with age.

Pell and D'Alonzo[6] have shown that, of 209 persons (age range 25-64 years) employed at the Du Pont Company, who developed myocardial infarction during a 1-year period, the most striking factor was the difference in sex incidence, which was 0·2 cases per 1,000 female employees and 2·9 cases per 1,000 male employees. The ages of the 2 female patients were 60 and 64 years respectively.

Current investigations in many countries show a pronounced difference between the two sexes in mortality from myocardial ischaemia in the 40-59 year age groups, males showing a considerably higher mortality than females.

This sex difference between the incidence and mortality rates has given rise to much speculation and has led to the theory that in females some endocrine sex factor may exert a protecting effect, which may operate until after the menopause, although to a diminishing degree. Keys[7] though, in a recent symposium on arteriosclerosis, in discussing the role played by non-dietary factors in the aetiology of atherosclerosis, suggested that even the sex factor may have its limitations. He showed that in population groups with a relatively low, compared with a high death rate from the disease, the sex ratio tends to become narrower. One of us (I.B.)[8] has inferred from the above that among the South African Bantu, where the incidence of coronary thrombosis is almost negligible, the sex difference may well be no longer apparent. We suggested the possibility that it required a particular environmental (dietary and non-dietary) context, highly promotive of mortality from coronary artery disease, to 'light up' the sex factor; the latter not being apparent in a context which promotes a low death rate from the disease.

The above observations are borne out in a recent WHO report on *A Study Group of Atherosclerosis and Ischaemic Heart Disease*. The report states, *inter alia*, that 'in populations where ischaemic heart disease is frequent, as in the USA, the ratio of male to female deaths from ischaemic heart disease is very high in young persons and diminished after the menopause, whereas in countries such as Italy and Portugal the disparity in frequency is smaller'. This report further states that the mortality from coronary artery disease in married women in the age group 45-49 years is greater in those with no children than in those having children.[9]

One of the reasons why a small number of experienced cardiologists maintain that at the present time there is no marked sex bias in the incidence of coronary disease is that the clinical course of ischaemic heart disease tends to be somewhat different in the two sexes—in the male the onset and course is more sudden and dramatic and with a rapidly fatal outcome. This might account for the fact that recent hospital surveys in Sweden and the USA (and possibly in South Africa) show more female patients than might be expected from the mortality statistics of the regions concerned.[9]

Experimental and clinical evidence suggests that oestrogens may play an important role in the relative immunity from coronary artery disease in the premenopausal woman, and that oestrogen therapy to the atherosclerotic male favourably alters an abnormal lipoprotein pattern to that of a normal one. Thus, Katz and his co-workers have shown that the administration of oestrogenically active compounds (orally or parenterally) to cholesterol-fed cockerels results in an increase in the ratio of free to total plasma cholesterol, in enhanced hyperphospholipaemia with a lowering of the cholesterol phospholipid ratio, and in a reduction in the alpha-lipoprotein and Sf 20-100 levels and that it prevents coronary atherogenesis without influencing the formation of aortal lesions. Katz further quotes work done in his laboratories on cockerels in which he shows that the coronary vessels are practically free of lesions when oestrogens and an atherogenic diet are administered simultaneously, and that oestrogen reverses both the lipophage and fibroblastic components of coronary atherosclerotic plaques. Katz and his co-workers have also shown that sexually mature chickens, unlike immature birds but like sexually mature humans, exhibit a

significant sex susceptibility to coronary atherosclerosis.[10-14]

Barr[15] has shown that the administration of oestrogen to men who have survived one or more myocardial infarctions, results in the restoration to normal of the usually gross abnormal lipid disturbances found in atherosclerosis; i.e., oestrogen administration results in an increase in alpha-lipoprotein levels with a corresponding decrease in the beta fraction, a reduction in serum-cholesterol levels, and a marked improvement in the cholesterol-phospholipid ratio of serum and beta lipoprotein fraction. Women, too, who have survived a myocardial infarction, show similar improvement when treated with oestrogens. The effects of androgen therapy on the lipid picture are in the main exactly opposite to the beneficial effects of oestrogens.

Eilert[16] has shown that increased oestrogen levels depress plasma cholesterol, and Boyle[17] has reported reduction in all serum lipids, including cholesterol, in humans during long-term administration of oestrogens.

Wuest *et al.*[18] reported that in oophorectomized patients coronary sclerosis, decade for decade, was of a greater degree of severity than in a control group and conclude that ovarian hormones exert at least one of the influences in the development of atherosclerosis.

Cooper [19] has shown that under the influence of oestrogens the lipoprotein pattern as well as the atherogenic index improved remarkably, whilst Gitman[20] has shown similar effcts following a single intravenous injection of oestrogen.

Gertler *et al.*[21] have shown a paucity of atherosclerosis in eunuchs and a greater liability of patients with predominant 'maleness' to myocardial infarction, whilst Cooper[19] has shown that castrated males, eunuchs and eunuchoids have lipoprotein patterns similar to those observed in healthy young females.

It has been shown by Oliver and Boyd[22] that changes in plasma lipids occur during the menstrual cycle. A striking fall in the plasma cholesterol esters, a less marked decrease in phospholipids and thus a decrease in the cholesterol-phospholipid ratio, occurred at the time of ovulation and immediately before menstruation, and they suggested that the point where the plasma cholesterol-phospholipid ratio is lowest coincides with the phase in the menstrual cycle where oestrogen activity is maximal. One of us (I.B.) has shown similar findings, namely a decrease in total cholesterol due mainly to a decrease in the esterified fraction and a far smaller decrease in the serum phospholipid at the mid-menstrual cycle, i.e. at the time of ovulation. It has also been shown at this Institute that oestrogens administered in small doses to male hypercholesterolaemic patients who had had a myocardial infarction resulted in a lowered cholesterol phospholipid ratio.[23]

Oliver and Boyd[2] and Steiner *et al.*[24] have also shown that ethinyl oestradiol is effective in improving the abnormal lipoprotein patterns which occur in clinical coronary artery disease. Ethinyl oestradiol was the most potent oestrogen in this respect, although hexestrol and oestradiol produced similar trends. Oestrone and oestriol failed to produce any significant alterations in the lipid picture when administered in doses of equivalent oestrogenic potency.

Boyle[25] treated a group of patients suffering from proved coronary artery disease and a group of hypercholesterolaemic patients suffering from xanthomatosis tuberosum and/or tendinosum with varying doses of ethinyl oestradiol. This treatment resulted in a reduction in all serum-lipid levels—a decrease in the cholesterol-phospholipid ratio, a marked decrease in the beta lipoprotein levels and an increase in the alpha level.

From the above review two facts emerge:

1. That a marked sex difference in the incidence of coronary thrombosis exists.

2. That experimental and clinical observations suggest that oestrogen administration rapidly reverts abnormal lipid patterns to relatively normal ones.

A COMPARATIVE STUDY

One of the most commonly recognized factors, in the aetiology of coronary thombosis to date is the broad interrelationship between dietary fats, lipid disturbances, and the development of atherosclerosis. However, since men and women under the age of 50 consuming almost identical diets show such marked differences in the incidence of coronary atherosclerosis, it was felt that a comparative study on urinary oestrogen levels in a group of normal male subjects and in a similar group, matched for age, of male patients who had recently suffered from a coronary occlusion, might be profitable.

We have previously suggested that alterations in the balance between male and female sex hormones may well be involved in the observed differences between cholesterol levels in the South African Bantu and White subjects, and indirectly in the very low incidence of coronary thrombosis in the former.[26,27]

We have already shown that total oestrogens as well as the very active and potent oestradiol fraction is significantly higher in the young male Bantu compared with young White subjects.[27] If oestrogen levels play a role in the aetiology of coronary atherogenesis then an entirely different oestrogen pattern should be found (on comparing oestrogen levels) in a group of subjects who have recently experienced a myocardial infarction when compared with a similar group who clinically show no evidence of coronary artery disease. Such a comparative study forms the basis for this investigation.

We obtained 24-hour specimens of urine from 20 European males subjects employed at the South African Institute for Medical Research and who were in excellent health, and from 23 European male subjects who were patients at the Johannesburg General Hospital. All the latter had been hospitalized for a recent acute myocardial infarction. This diagnosis was based in each case on clinical evidence and confirmed by ECG.

The age ranges and mean ages for the normal group was 44-65 years (mean 55) and for the myocardial infarction group 42-81 years (mean 57) respectively.

The technique used for estimating total oestrogen, oestradiol, oestrone and oestriol was that described by Brown[28] and identical with that used by us when we compared urinary oestrogen levels in normal young male urbanized South African Bantu and young normal White subjects.[27]

RESULTS

Tables I and II show the amounts of total oestrogen, and the absolute and relative amounts of the 3 fractions oestradiol, oestrone and oestriol, which we found in 24-hour specimens of urine from the normal and coronary artery disease subjects respectively.

The mean total urinary oestrogen value in the normal European (mean age 55) was 14·4 μg. as compared with 10·8 μg. in the acute myocardial infarction group (mean age 57).

The mean oestradiol value was 2·1 μg. in the healthy sub-

TABLE I. ANALYSIS OF THE OESTROGEN CONTENT OF 24-HOUR SPECIMENS OF URINE IN NORMAL EUROPEAN MALE SUBJECTS (AVERAGE AGE 55)

Age	Oestradiol µg.	Oestradiol %	Oestrone µg.	Oestrone %	Oestriol µg.	Oestriol %	Total Oestrogen µg.
45	2·5	18·1	4·9	35·5	6·4	46·4	13·8
60	3·3	22·0	8·7	58·0	3·0	20·0	15·0
65	0·8	4·8	8·4	50·3	7·5	44·9	16·7
52	0·9	6·0	8·1	53·6	6·1	40·4	15·1
60	3·4	19·1	9·0	50·6	5·4	30·3	17·8
48	1·1	9·3	5·4	45·8	5·3	44·9	11·8
61	0·5	4·7	3·5	33·0	6·6	62·3	10·6
57	1·6	9·1	11·3	64·6	4·6	26·3	17·5
57	0·9	5·2	3·5	20·5	12·7	74·3	17·1
44	1·0	17·9	3·3	58·9	1·3	23·2	5·6
48	1·7	8·1	12·5	59·2	6·9	32·7	21·1
54	3·5	21·3	6·2	37·8	6·7	40·9	16·4
47	3·5	21·6	7·2	44·4	5·5	34·0	16·2
58	3·8	23·7	4·8	30·0	7·4	46·3	16·0
57	3·0	17·9	6·4	38·1	7·4	44·0	16·8
52	2·1	13·1	6·4	40·0	7·5	46·9	16·0
53	1·7	14·4	5·2	44·1	4·9	41·5	11·8
59	2·8	28·6	3·4	34·7	3·6	36·7	9·8
65	1·2	18·7	2·8	43·8	2·4	37·5	6·4
58	2·9	17·3	5·7	33·9	8·2	48·8	16·8
Mean: 55	2·1	15·0	6·3	43·8	6·0	41·2	14·4

TABLE II. ANALYSIS OF THE OESTROGEN CONTENT OF 24-HOUR SPECIMENS OF URINE IN EUROPEAN MALE SUBJECTS SUFFERING FROM ACUTE MYOCARDIAL INFARCTION (AVERAGE AGE 57)

Age	Oestradiol µg.	Oestradiol %	Oestrone µg.	Oestrone %	Oestriol µg.	Oestriol %	Total Oestrogen µg.
48	2·0	26·3	1·5	19·8	4·1	53·9	7·6
64	2·5	16·6	7·9	52·3	4·7	31·1	15·6
55	0·1	2·2	1·3	28·2	3·2	69·6	4·6
47	0·8	5·1	5·1	32·7	9·7	62·2	15·6
58	0·9	8·4	3·0	28·0	6·8	63·7	10·7
48	0·0	0·0	9·6	82·8	2·0	17·2	11·6
43	1·9	11·5	4·2	25·5	10·4	63·0	16·5
64	0·3	6·7	1·9	42·2	2·3	51·1	4·5
52	0·1	3·7	0·7	25·9	1·9	70·4	2·7
71	0·0	0·0	3·0	30·9	6·7	69·1	9·7
48	0·5	10·2	0·6	12·2	3·8	77·6	4·9
42	1·2	12·1	5·2	52·5	3·5	35·4	9·9
51	1·7	13·9	6·0	49·2	4·5	36·9	12·2
66	0·4	2·3	6·0	33·3	11·6	64·4	18·0
68	0·0	0·0	2·8	32·6	5·8	67·4	8·6
46	0·2	2·2	2·2	23·7	6·9	74·1	9·3
65	0·0	0·0	4·8	51·6	4·5	48·4	9·3
80	0·0	0·0	2·4	23·1	8·0	76·9	10·4
81	0·0	0·0	1·1	6·4	16·2	93·6	17·3
43	0·2	2·3	3·3	38·4	5·1	59·3	8·6
56	2·2	9·2	6·9	29·0	14·7	61·8	23·8
60	0·0	0·0	3·3	33·0	6·7	67·0	10·0
56	1·9	18·5	4·3	41·7	4·1	39·8	10·3
Mean: 57	0·7	6·5	3·7	34·6	6·4	58·9	10·8

jects as compared with 0·7 µg. in the patients: whilst the percentages of oestradiol to total oestrogen were 15% and 6·5% respectively.

The mean oestrone value was 6·3 µg. with a percentage of 43·8% in the healthy group as against 3·7 µg. and 34·6% in the myocardial infarction group.

The mean oestriol value was 6 µg. and 41·2% in the normal group and 6·4 µg. and 58·9% in the patients.

These results, when subjected to statistical analysis, show the following:

1. A very highly significant decrease in the total oestrogen level per 24-hour specimen of urine in the coronary patient when compared with the normal European group ($p = < 0·005$).

2. A very highly significant decrease in the absolute oestradiol level ($p = < 0·005$) and a very highly significant decreased percentage of oestradiol in the coronary group ($p = < 0·005$).

3. A very highly significant decrease in the absolute oestrone level ($p = < 0·005$) and a statistically significant decreased percentage in the coronary group ($0·01 < p < 0·025$).

4. No significant differences in the absolute oestriol values in the 2 groups but a very highly significant *increased* percentage of oestriol was found in the coronary group ($p = < 0·005$).

When the values obtained by us in the group of European subjects of a mean age of 55 are compared with the values previously obtained by us in Europeans of a younger age group (mean 31 years) we find that the total oestrogen,

absolute oestradiol, oestrone and oestriol levels are statistically higher in the former group. No significant differences were observed in the percentage of oestradiol, whilst the oestrone percentage was statistically lower and the oestriol percentage significantly higher in the older age group.

DISCUSSION

The oestrogenic steroids are oestradiol, oestrone and oestriol. The oestradiol fraction may be regarded as the true oestrogenic hormone and the oestrone and oestriol fractions as breakdown products. Oestradiol and oestrone are interconvertible and both these steroids can be excreted as oestriol (the relatively inert fraction). Alpha oestradiol is approximately 12 times more potent than oestrone and at least 80 times more potent than oestriol.

Our results show that not only is the total urinary oestrogen level lower in the myocardial infarction group but that the potent oestradiol fraction is significantly lower (both relative and absolute) in this group and the inert fraction oestriol is significantly higher.

Bauld *et al.*[29] carried out a preliminary investigation in the metabolism of oestrogen in men with myocardial infarction. The technique for oestrogen determination was the same as used by us: 400 μg. oestradiol 17 β in oil were injected intramuscularly in a control group of normal men and in a group of male subjects who had experienced a previous myocardial infarction. The total amount of oestrogen excreted after the injection was similar in the two groups but there was a marked difference in the relative proportions of oestriol and oestrone. The urinary oestriol: oestrone ratio was significantly higher in the infarction group than in the control group. These findings were unrelated to age or to the interval after the infarction. The findings of Bauld and his co-workers suggest a disturbed metabolism of oestrogen in those who have had a previous myocardial infarction.

If we compare the ratio of oestriol to oestrone in the two groups studied by us, we find in the coronary artery disease group a ratio of 1·7 : 1 and in the control group a ratio of 0·94 : 1. These findings substantiate those of Bauld *et al.*

When we compare the ratio of the most potent fraction oestradiol to the least active fraction oestriol a ratio of 0·11 is obtained in the coronary group as against a ratio of 0·36 in the control group. Table III shows the ratio of oestradiol to oestriol obtained by us in a group of young Bantu urban

TABLE III. RATIO OF OESTRADIOL TO OESTRIOL IN URBANIZED BANTU AND EUROPEAN MALE SUBJECTS (MEAN AGE 31 YEARS), IN NORMAL MALE EUROPEANS (MEAN AGE 55), AND IN MALE CORONARY PATIENTS (MEAN AGE 57)

Bantu (*mean age* 31)	*European* (*mean age* 31)	*European* (*mean age* 55)	*Coronary Group* (*mean age* 57)
0·71	0·43	0·36	0·11

males and young normal European male subjects compared with the ratios obtained in this investigation. These findings show that the metabolism of oestrogens in patients suffering from coronary artery disease differs markedly from that found in young healthy subjects who are relatively immune from coronary occlusion.

The metabolism in the coronary group seems to be markedly accelerated, yielding a much higher percentage of oestriol with a consequent alteration of the ratio of the active to the inactive fractions. In the Bantu, where the incidence of coronary artery disease is very low, a retarded breakdown of oestradiol is found, and in normal European subjects the metabolism is intermediate between that of the Bantu and the diseased group.

Marmosston *et al.*,[30] using a biological technique for oestrogen determination, compared urinary oestrogen levels in postmenopausal women with and without evidence of myocardial infarction: 55% of the women in the control group excreted 0·50μg. or higher oestradiol as compared with 22% in the infarcted group. The control group excreted 0·516 μg. equivalents of oestradiol as against 0·244 μg. in the myocardial infarction group. These findings, too, suggest an altered metabolism of oestrogen in coronary atherosclerosis and they confirm our findings.

Stamler *et al.*,[31] in an interim report on the effects of oestrogen therapy in males with a history of a previous single myocardial infarction, show that in a placebo-treated group of 26 patients under treatment for 2 months or longer a second infarction occurred in 6, with 3 fatalities, whilst in 39 subjects under oestrogen therapy for a similar period no recurrences occurred. Despite the short duration of treatment and the small numbers treated, it was felt that continuation and expansion of this investigation was warranted.

Our present together with our previous findings, when taken in conjunction with available experimental and clinical evidence, tend to support the thesis that the gonadal steroid-play an important role in the aetiology of coronary atheros genesis.

The different patterns of metabolism of oestrogens in the Bantu, when compared with the myocardial group, conceivably plays an important role in the almost non-existence of coronary thrombosis in the Bantu. Many factors could conceivably be responsible for the altered hormonal pattern, and it is even possible that the type of diet consumed by them directly or indirectly influences their endocrine system and may in part be responsible for it; in fact, unpublished work carried out in our laboratories seems to suggest that European subjects when habituated to the 'Bantu type of diet' show an oestrogen pattern very similar to that of the Bantu.

The role played by oestrogens in the prevention and therapy of coronary artery disease needs further intensive study, but the comparative immunity of premenopausal women to coronary thrombosis, the different sex incidence, and the rarity of coronary thrombosis in the Bantu, suggest that the gonadal steroids may be of vital significance in the development of coronary atherosclerosis.

SUMMARY

1. Experimental and clinical evidence suggesting that oestrogens play a role in coronary atherosclerosis are reviewed.

2. Urinary oestrogen levels in 20 normal European middle-aged male subjects and 23 European middle-aged hospitalized subjects suffering from a recent myocardial infarction were determined.

3. Highly significant decreases in the total oestrogen level were found in the myocardial infarcted group.

4. Decreased absolute and relative oestradiol and oestrone levels and a highly significant increased relative level of oestriol were found in the coronary group.

5. It was suggested that the metabolism of oestrogens is accelerated in the coronary group, that a retarded breakdown

of oestradiol is found in the Bantu, whilst the normal European occupies an intermediate position.

6. The possible role played by a particular diet on oestrogen metabolism is discussed.

7. The suggestion is made that the gonadal steroids are important in the development of coronary atherosclerosis.

REFERENCES

1. Smit, Z. M. (1957): S. Afr. J. Lab. Clin. Med., 3, 29.
2. Oliver, M. F. and Boyd, G. S. in Minnesota Heart Association (1955): *Arteriosclerosis*, p. 64. Rochester: University of Minnesota.
3. Hueper, W. C. (1956): Amer. J. Clin. Path., 26, 559.
4. Martin, W. J. (1956): Brit. Med. J., 1, 1523.
5. Lee, K. T. and Thomas, W. A. (1956): Arch. Intern. Med., 97, 421.
6. Pell, S. and D'Alonzo, G. A. (1958): J. Amer. Med. Assoc., 166, 332.
7. Keys, A. in Minnesota Heart Association (1955): *op cit.*[3]
8. Walker, A. R. P., Andersson, M. and Bersohn, I. (1956): Brit. Med. J., 1, 1234.
9. Wld. Hlth. Org. Tech. Rep. Ser. (1957): No. 117. *Study Group on Atherosclerosis and Ischaemic Heart Disease.*
10. Katz, L. N., Stamler, J. and Pick, R. (1955): *Symposium on Atherosclerosis*, p. 236. United States National Research Council.
11. Pick, R., Stamler, J., Rodbard, S. and Katz, L. N. (1952): Circulation (N.Y.), 6, 276.
12. *Idem* (1952): *Ibid.*, 6, 858.
13. Stamler, J., Pick, R. and Katz, L. N. (1953): Circulat. Res., 1, 94.
14. *Idem* (1954): Circulation (N.Y.), 10, 251.
15. Barr, D. P. in Minnesota Heart Association (1955): *op. cit.*[3]
16. Eilert, M. L. (1949): Amer. Heart J., 38, 472.
17. Boyle, E. (1954): Circulation (N.Y.), 10, 587.
18. Wuest, J. H., Dry, T. J. and Edwards, J. E. (1953): *Ibid.*, 7, 801.
19. Cooper, E. E. (1956): Amer. Practit., 7, 436.
20. Gitman, I. and Greenblatt, I. J. (1953): Angiology, 4, 502.
21. Gertler, M. M., Garn, M. S. and White, P. D. (1951): J. Amer. Med. Assoc., 147, 621.
22. Oliver, M. F. and Boyd, G. S. (1953): Clin. Sci., 12, 217.
23. Bersohn, I. (1956): A.R. S. Afr. Inst. Med. Res.
24. Steiner, A., Payson, H. and Kendall, F. E. (1955): Circulation (N.Y.), 11, 784.
25. Boyle, E. (1954): *Ibid.*, 10, 587.
26. Bersohn, I. and Wayburne, S. (1956): Amer. J. Clin. Nutr., 4, 117.
27. Bersohn, I. and Oelofse, P. J. (1957): S. Afr. Med. J., 31, 1172.
28. Brown, J. B. (1955): Biochem. J., 60, 185.
29. Bauld, W. S., Milne, I. G. and Givner, M. L. (1956): J. Clin. Invest., 35, 689.
30. Marmosston, J., Hoffman, O., Sobel, H. and Starr, P. in Minnesota Heart Association (1955): *op. cit.*,[3]
31. Stamler, J., Pick, R. and Katz, L. N. (1954): Circulation (N.Y.), 10, 587.