Studies on the Distribution and Excretion of Mercury, Gold, and Lead under the influence of dithiols.

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INTRODUCTION

The purpose of these studies has been to attempt to clarify the mode of action of dithiols when administered to animals poisoned with gold, mercury, or lead. For an understanding of the problems involved, it is necessary to review briefly, the evidence which led to the introduction of dithiols as antidotes to poisoning by first, arsenic, and secondly, various other metals.

After the identification of thiol groups in living cells, Ehrlich suggested, in 1909, that arsenic combined with the thiol groups of tissue constituents, and exerted its toxic effects as a result of this combination. Direct evidence in favour of this view was provided by Walker (1924, quoted by Stocken and Thompson, 1946a), who investigated the action of arsenicals on dried muscle powder. He found that the arsenicals caused a progressive inhibition of certain catalytic effects shown/

shown by this preparation, and that it was accompanied by a disappearance of protein thiol groups. Rosenthal (1932) established that trivalent arsenicals did combine with the thiol groups of tissue proteins.

Many important enzymes depend on free thiol groups for their activity (Hellermann, 1937; Barron and Singer, 1943; Peters et al., 1946). One enzyme in particular, pyruvate oxidase, was found to be markedly inhibited by arsenic (Peters, 1936; 1937). Peters suggested that this enzyme contained essential thiol groups; and this was supported by the fact that iodoacetate, which was known to combine with thiol groups (Dickens, 1933), is also a potent inhibitor of pyruvate oxidase. Eventually, the existence of essential thiol groups in this enzyme was demonstrated (Barron and Singer, 1945; Peters and Wakelin, 1946). Present knowledge of the central position of this enzyme in cell metabolism makes it obvious that any inhibition would cause widespread effects.

Meanwhile, Voegtlin and his associates had found that simple monothiols, such as cysteine and reduced glutathione, could diminish the trypanocidal and/

and toxic effects of oxophenarsine or sodium arsenite (Voegtlin et al., 1923, 1925; Rosenthal and Voegtlin, 1930). It was also shown that the reduction in oxygen consumption of animal tissues caused by oxophenarsine or sodium arsenite, could be prevented by the addition of glutathione (Voegtlin et al. 1931). In all these experiments, however, a large excess of the monothiol was required in order to antagonize the action of the poison. Glutathione is also capable of abolishing the anti-spirochaetal action of oxophenarsine, but again a large excess of glutathione is required (Eagle, 1939).

Schmitt and Skow (1935), investigating the action of arsenite on nerves, found that glutathione and cysteine could delay the extinction of the nerve action potential by arsenite; but even high concentrations of monothiols could not prevent the eventual extinction, nor could they reverse it once it was established.

Lewisite was found to be a more potent inhibitor of pyruvate oxidase than were any of the therapeutic arsenicals, and monothiols were unable to afford/

afford any protection against this substance (Peters et al. 1946; Stocken and Thompson, 1946b).

Cohen, King and Strangeways (1931), studying the reactions between aromatic arsenicals and thiols, found that the equilibrium -

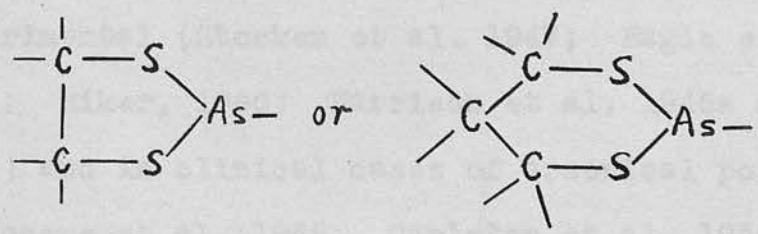
$$RAsO + 2R^1SH \rightleftharpoons RAs(SR^1)_2 + H_2O$$

is shifted to the left as the pH increases. Excess of thiol favours the opposite reaction. However, Strangeways (1937) showed that, in dilute solutions, hydrolysis of thioarsinites predominates, and the protection afforded by glutathione against oxophenarsine becomes less complete with progressive dilution.

Thus it was evident that monothiols could afford some degree of protection against oxophenarsine, but not against the more toxic arsenicals. It seemed probable that this variation in activity was due to differences in the degrees of dissociation of the respective thioarsinites.

The advent of the war in 1939 initiated an investigation, under the direction of Peters, into the actions of arsenical war-gases. The reactions of arsenicals with proteins were investigated, using the/

the thiol-containing protein, kerateine. Analyses of the arsenical-kerateine complexes showed that the arsenic had combined solely with thiol groups, and that most of the arsenic appeared to be in combination with two adjacent thiol groups, forming a ring structure (Stocken and Thompson, 1946a). This evidence suggested that, in order to remove arsenic from protein, a dithiol, capable of forming an even more stable cyclic thioarsinite, would be required. 1 : 2,- or 1 : 3,-substituted dithiols were the obvious choice, since these would form five- or six-membered rings:-



It may be noted that support for this concept was given later by Whittaker (1947), who synthesized a series of dithiols, and found that those forming such rings had the highest antidotal activity.

Several dithiol compounds were prepared and tested for their ability to protect the pyruvate oxidase/

system against inhibition by lewisite and to cause survival of animals contaminated with lewisite. The dithiols were generally quite successful in these tests, but one, 2,3-dimercaptopropanol, was considered more suitable for therapeutic purposes because of its physical properties and lower toxicity. It was therefore subjected to further testing as an antidote to lewisite or arsenite poisoning in animals, and then for its ability to prevent vesication by lewisite in man. The results of all these experiments indicated a high antidotal activity (Stocken and Thompson, 1946b). This conclusion was completely confirmed in later studies, both in experimental (Stocken et al. 1947; Eagle et al. 1946; Riker, 1946; Harrison et al. 1946a and b; etc.) and in clinical cases of arsenical poisoning (Longcope et al. 1946; Carleton et al. 1946; Eagle and Magnuson, 1946; Carleton et al. 1948; etc.). 2,3-dimercaptopropanol was named British Anti-Lewisite (B.A.L.) during the war, but since then has been termed dimercaprol. It is a fairly toxic substance, and this has prompted investigation of related compounds for their anti-arsenical activity and/

and toxicity. Besides those substances tested by Stocken and Thompson (1946b), a number of other dithiols have been studied (Chenoweth et al. 1946; Fitzhugh et al. 1946; Weatherall, 1949). So far, only one substance, the glucoside of dimercaprol (BAL-Intrav), has been shown to possess any advantages over dimercaprol itself (Danielli et al. 1947). This substance is much less toxic than dimercaprol and possesses considerable anti-arsenical activity, though it does not appear to be as effective as dimercaprol when administered after poisoning with arsenic has been established (Weatherall, 1949). When the effectiveness of dimercaprol in arsenical poisoning had been demonstrated, investigations were extended to poisoning by other metals. Many heavy metals readily form mercaptides, and a number react with dimercaprol, giving almost insoluble, coloured compounds (Barron et al. 1947).

In the case of mercury, previous observations tended to support the view that compounds of this metal reacted with essential thiol groups in enzymes (Hellermann, 1937). Eagle (1939) obtained reversal of the spirochaeticidal action of mercury by glutathione;/

glutathione; and the latter substance prevented the inhibition of growth of Esch coli by mercuric chloride (Fildes, 1940). Glutathione can also reverse the inhibition of the pyruvate oxidase system of gonococci caused by p-chloromercuri-benzoic acid (Barron and Singer, 1945).

Thompson and Whittaker (1947) found that both dimercaprol and glutathione could protect the pyruvate oxidase system of brain from inhibition by mercuric chloride, but Barron and Kalnitsky (1947), using the enzyme succinoxidase, reported that dimercaprol was definitely superior to glutathione.

The results of a large number of animal experiments have shown that dimercaprol can counteract the toxic effects of mercuric chloride (Braun et al. 1946; Gilman et al. 1946a; Ginzler, 1946; Stocken, 1947; Graham and Hood, 1948), and of organic mercurials (Long and Farah, 1946; Handley and La Forge, 1947; Sussmann and Schack, 1947). It has also been found that dimercaprol glucoside is more effective than dimercaprol in preventing the toxic effects of mercuric chloride, by Harrison and Randoll (1948), using a tissue-culture technique; and/

and by Gilman et al. (1946a), studying the acute poisoning of rabbits and dogs.

Clinical reports have confirmed the effectiveness of dimercaprol therapy in acute mercuric chloride poisoning (Longcope and Luetscher, 1946 and 1949).

Gold salts inhibit the pyruvate oxidase system, and this action can be prevented by dimercaprol (Thompson and Whittaker, 1947). Dimercaprol decreases the toxicity of gold sodium thiosulphate in mice (Swanson et al. 1947), and can increase the survival-time and growth-rate of rats poisoned with certain gold salts, but the dimercaprol-gold complex appears to have an injurious effect on the kidney (Levey and Smyth, 1947). However, Kuzell et al. (1948) reported that dimercaprol could protect rats against a lethal dose of gold sodium thiosulphate injected intramuscularly, but not against a lethal dose of gold chloride given by the intraperitoneal route. Clinical reports have indicated a favourable response to dimercaprol therapy in cases of toxic reactions to gold (Cohen et al. 1947; Lockie et al. 1947; Margolis and Caplan, 1947; Ragan/

Ragan and Boots, 1947; etc.).

The treatment of experimental lead poisoning by dimercaprol has produced much conflicting evidence. Braun et al. (1946) found that dimercaprol increases the mortality rate of rabbits injected intraperitoneally with lead nitrate, but decreases the mortality after intravenous injection of lead acetate (Braun et al. 1949). On the other hand, Germuth and Eagle (1948) reported that dimercaprol failed to protect rabbits after intravenous or subcutaneous injection of lead acetate, though the excretion of lead was increased. The latter finding was also reported by Ginsburg and Weatherall (1948). Weatherall (1948) showed that dimercaprol had little effect on the mortality of mice receiving repeated intraperitoneal injections of lead acetate, but dimercaprol glucoside caused a significant reduction in mortality. He also found that the anaemia of acutely lead-poisoned rabbits could be diminished by both dithiols. The nephrotoxic action of lead in rats can be prevented by dimercaprol (Chiodi and Sammartino, 1948). However, dimercaprol has little effect in increasing the excretion of lead in more chronic/

chronic phases of poisoning in rabbits (Adam et al. 1949) or mice (Anderson, 1949).

In clinical cases of lead poisoning, dimercaprol therapy has been found to increase the urinary excretion of lead, but conflicting opinions have been given of its possible therapeutic value (Ryder et al. 1947; Telfer, 1947; Longcope and Luetscher, 1949).

Dimercaprol has also been reported to reduce the mortality of animals poisoned with cadmium (Gilman et al. 1946b; Tobias et al. 1946; Harrison et al. 1947), antimony (Braun et al. 1946; Eagle et al. 1947), bismuth, chromium and nickel (Braun et al. 1946), tungsten and copper (Braun et al. 1949). Dimercaprol is stated to be ineffective in poisoning by iron (Edge and Somers, 1948), silver (Olcott and Riker, 1947), thallium and selenium (Braun et al. 1946), vanadium (Braun et al. 1949) and uranium (Macnider, 1948; Braun et al. 1949). These conclusions are by no means unanimous. For instance, Gammill et al. (1947) found that dimercaprol increased the toxicity of various antimonials; Graham and Hood (1948) reported that dimercaprol had a deleterious/

deleterious effect in poisoning by bismuth. It seems likely that these findings are largely conditioned by the methods of poisoning and treatment.

The following studies of the effects of dimercaprol or dimercaprol glucoside on the distributions of mercury, gold and lead, were undertaken in an attempt to clarify their antidotal actions. Similar work has been carried out by Tepperman (1947) in the case of cadmium, by Ginsburg and Weatherall (1948) in the case of lead, and by Bruner (1950) for zinc.

These experiments also provided an opportunity for a fresh examination of the distributions of mercury and gold.

METHODS

Materials:- The dimercaprol was a water-purified sample, and was stored in a refrigerator when not in use. Solutions in 66% (v/v) aqueous propylene glycol were made up freshly as required.

Dimercaprol glucoside was received as the barium salt, contained in nitrogen-filled ampoules. Solutions of the free dithiol were obtained by adding sulphuric acid to an aqueous suspension of the barium salt, and centrifuging off the barium sulphate. Complete removal of the barium was ascertained by observing the absence of a precipitate after the addition of saturated sodium sulphate solution, and the solution of the dithiol was neutralized with sodium hydroxide. All dimercaprol glucoside solutions were made up freshly immediately before use.

The impurity of the preparations of dimercaprol glucoside necessitated estimations of the thiol content of each solution, by means of iodine titration. N/10 iodine was titrated against the dithiol in the presence/

presence of N. hydrochloric acid, and at a temperature of 0°C. A less sharp end-point is obtained if the titration is carried out at room-temperature. All doses of the glucoside were calculated from the estimated thiol contents. This procedure gave slightly variable results, but is probably more satisfactory than calculating the dosage from the weight of barium salt used (Weatherall, 1949).

1:3-dimercaptopropanol was synthesized by Dr. L.A. Stocken, and 1:4-dithioerythritol by Dr. L. N. Owen. Both substances were of 95-100% purity, and were dissolved in water for administration.

The parathyroid extract used in the sub-acute distributions of lead was a commercial preparation ("Parathormone" Lilly).

The radio-active isotopes of gold and mercury (Au^{198} and $Hg^{203-205}$) were obtained by neutron irradiation of the respective chlorides at the Atomic Energy Research Establishment, Harwell. The irradiation caused the formation of radio-active chloride (Cl^{36}) as well, and it was necessary to remove the latter in order to prevent interference with the estimation of radio-activity due to the metal/

metal alone. This removal was carried out by dissolving the salt in water, and reducing it to the metal with stannous chloride in the case of mercury, and with ferrous sulphate in the case of gold. The metal was centrifuged off, and the supernatent liquid discarded. After washing with water, the metal was dissolved in aqua regia, and gently heated almost to dryness. Drying was completed in a vacuum desiccator.

In the case of gold, solutions for injection were prepared by adding a trace of the isotope to solutions of gold chloride. The poor specific activity of the mercury necessitated its quantitative transfer to injection solutions.

One radio-active isotope of lead (Thorium B, Pb^{212}) was obtained from the deposit on a charged button exposed to a thorium source. The button was heated gently in a slightly acidified 0.01% (w/v) solution of lead acetate, so that the Pb^{212} exchanged for part of the lead in solution. Further lead acetate was then added according to the desired dosage. The other isotope (radium D, Pb^{210}) was obtained from an equilibrium mixture of radium D, E, and/

and F, by displacing the radium E and F with nickel, added as foil to a solution in hydrochloric acid. A small quantity of the resulting solution of radium D was added to a solution containing lead acetate, so that the final solution contained 2.07mg. of lead and 2-3 microcuries of radium D per ml.

The concentrations of gold, mercury, or lead solutions were adjusted so that a volume of 1.0ml. was injected per kg. of body weight. The solutions were rendered isotonic with dextrose, and were neutralised before injection. Solutions of mercuric chloride tended to be rather acid, however, as the further addition of alkali caused the formation of insoluble basic chlorides.

The relevant physical properties of these isotopes are summarised in Table 1.

Table 1/

Table 1.

Isotope	Half-life	β-radition	γ-radiation	Approx. activity per ml. of injection solution.
Au^{198}	2.7 days	0.96 Mev.	0.44 Mev.	200μC.
$Hg^{203-205}$	51.5 days	0.3 Mev.	0.28 Mev.	3-5μC.
Pb^{212}	10.6 hours	0.36 Mev.	-	200-400μC.
Pb^{210}	22 years	0.03 Mev.	0.05 Mev.	2-3μC.

ANIMALS:- Adult rabbits, of both sexes and various breeds, were used throughout these experiments. Injections of gold, mercury, or lead solutions were made into the marginal vein of one ear, each injection lasting 2-3 minutes. The animals were then placed in metabolism cages. Water was allowed ad libitum. For experimental periods lasting longer than 24-hours, food was also provided, and consisted of bran and oats, with cabbage every two or three days. Vessels containing food or water were placed outside the cage, so that no contamination of the urine or faeces could occur.

Injections of dithiols were made into the paravertebral muscles. The doses of dithiols were near the toxic range since maximal effects were being sought. The doses usually employed were 50mg./kg. and 12.5mg./kg. of dimercaprol, and 175mg./kg. and 55mg./kg. of dimercaprol glucoside. Expressed in terms of molar weights, these doses correspond approximately to 0.4mM./kg. and 0.1mM/kg. of dimercaprol, and 0.6mM./kg. and 0.2mM./kg. of the glucoside. Thus, a larger number of molecules of dimercaprol/

dimercaprol glucoside than of dimercaprol was administered. Injections of saline or propylene glycol were given to the control animals. At the end of the desired distribution period, the animals were killed by a blow on the occiput.

SAMPLING OF TISSUES:- Immediately after death, blood was collected by bleeding from the great veins into the thorax. Clotting was prevented by heparin, and the samples were centrifuged at once. Usually about 10ml. of blood was taken.

The organs were then dissected out taking care to avoid contamination between different tissues. Complete organs were washed with water, dried between filter-papers, and weighed. If the organ weighed less than 10g. it was ashed entire. In other cases, samples were taken from well-mixed choppings of the organ. Skeletal muscle samples were taken from the thigh, and bone samples from the long bones of one fore limb and one hind limb. Marrow was removed from diaphyses and estimated separately, and was washed, as far as possible, from samples of epiphyses. The injected ear, and the/

the piece of cotton wool used to control bleeding, formed a separate sample, so that the quantity of metal remaining at the site of the injection was known. The opposite ear was taken as a sample of skin.

Duplicate samples of liver and kidney were taken routinely, and provided an estimate of the error of the determinations.

ASHING OF SAMPLES:- After weighing, the tissues were placed in Kjeldahl flasks, containing 30ml. conc. nitric acid and 20ml. of 20% (v/v) perchloric acid, and were allowed to stand for several hours. The flasks were heated gently until the contents were boiling, taking care to avoid excessive foaming, and were then allowed to boil very gently until about 10ml. of solution remained. If this solution was not quite clear, a further 20ml. of nitric acid was added and boiling continued. Generally, it proved impossible to obtain a clear solution of faeces.

The solutions were then transferred to boiling-tubes, and diluted with water to a known volume, usually 50ml. Urine and bile were not subjected to/

to ashing, but were estimated directly. In the case of the sub-acute distributions of lead, samples were allowed to stand for at least five weeks to allow an equilibrium mixture of radium D and E to form, because the counter used was not sufficiently sensitive to detect the low-energy β-rays, emitted by the radium D, but counted the more energetic emission from radium E.

ESTIMATION OF RADIO-ACTIVITY:- The activity of the samples was measured by means of an M.R.C. Type 1 fluid Geiger-Muller counter. Preliminary experiments showed that the total count per minute, corrected for the background count, was linearly related to the amount of radio-active material present, in the case of each isotope.

Variations in the density of the solutions, within the range involved in the experiments, affected the counts of Pb^{212} and Pb^{210} negligibly. The radiations of $Hg^{203-205}$ and Au^{198} were absorbed to a greater extent, however, and graphs were drawn relating the decrease in count to the density of the solution. The density of each sample was measured/

measured by simple hydrometers, and the count of the sample corrected by reference to the appropriate graph.

From the counts obtained from aliquots of the injection solution, and the counts obtained from samples of tissues under similar conditions, the proportion of the dose in each sample, and hence the concentration of metal in the tissue, was calculated.

Although counts from such aliquots were made several times during each experiment, the shortness of the half-lives of Pb^{212} and Au^{198} necessitated a correction for the decay occurring during the counting of the samples of any one experiment. Each count was corrected by means of the formula,

$$N_0 = N_t . e^{\lambda t}$$

where N_0 = the number of atoms of isotope present initially

N_t = the number of atoms of isotope present after a time t

and λ = disintegration constant, which for Pb^{212} is 1.84×10^{-3} min.$^{-1}$, and for Au^{198} is 1.77×10^{-4} min.$^{-1}$

The/

The half-lives of $Hg^{203-205}$ and radium D were considered sufficiently long to neglect the effect of decay.

The proportion of the dose in any organ was calculated from the known weight of the organ, and, for the purpose of estimating the total recovery, the following approximate values for various tissues were used:- total blood volume - 70ml./kg.;

total weight of bone marrow - 20g./kg. (Nye,1931);

total weight of bone - 60g./kg.;

total weight of skin -120g./kg.; and

total weight of skeletal muscle -520g./kg. (Levine et al. 1941). The amounts in bone were calculated from the mean value for both diaphyses and epiphyses. This is not an accurate estimate and is useful only as a rough check that a reasonable fraction of the dose has been accounted for.

The standard error, obtained from the duplicate samples of liver and kidney, of the mercury estimations was $\pm$ 14%; of the acute lead estimations $\pm$ 4%; of the sub-acute lead estimations $\pm$ 12%; and of the gold estimations $\pm$ 8%.

RESULTS

Part 1: Mercury.

Results are presented for the distribution and excretion of mercury in twenty-eight rabbits, all of which received a single intravenous dose of mercuric chloride, either 5μM./kg. or 3.7μM/kg. and were killed after various periods of time. Some animals were treated with dimercaprol, and some with dimercaprol glucoside, at different times after the injection of mercuric chloride. The larger dose of mercury usually, and the lower dose occasionally, caused some degree of damage to the kidneys, as demonstrated by the presence of albumen and casts in the urine. As far as could be judged, this damage was less marked in treated animals. No other signs of toxic effects were noted, apart from general apathy, in the poisoned animals.

It should be noted that recoveries ranged from 70-90%. Preliminary recovery experiments showed that/

that as much as 50% of the mercury could be lost during ashing if the acid solutions were boiled vigorously. Allowing the solutions to evaporate under gentle heating reduced the loss to about 20%. As urine and bile were not ashed, mercury was not lost from these fluids in this way.

A further point of note is that variable quantities of mercury were left at the injection site, and in a few cases this amounted to as much as one-third of the dose. In these cases, the injections had been difficult due to blocking of the vein into which the injection was given, and this was probably the result of the acidity of the injection solutions. The mercury itself tends to irritate veins on intravenous injection, and may have contributed to the blockage.

Lastly, the error of the estimations was approximately $\pm$ 14%. This is higher than in the cases of lead and gold, and the increase is mainly if not entirely, due to the lower specific activity of the mercury. The time of counting samples was not increased sufficiently to attain the same accuracy as was obtained with the more active isotopes. These/

These three factors should be kept in mind when comparing results obtained in different animals.

In Tables 2 and 3, results are presented for rabbits which received 5μM./kg. (1.0mg. Hg./kg.) of mercuric chloride intravenously, and no further treatment. The concentrations of mercury in micrograms per gramme of tissue are shown in Table 2, and the percentages of the dose found in various organs and calculated for various tissues are shown in Table 3.

Although considerable variation occurred between rabbits receiving the same treatment, certain general trends can be seen. One hour after the injection of mercury, a small quantity of the element remained in the plasma; but within six hours, the amounts had fallen and were generally below the detectable level. Of all the organs and tissues sampled, the kidneys contained by far the highest concentration of mercury, and, after twenty-four hours, accounted for 20-30% of the mercury absorbed. The liver generally contained about 2μg. of mercury per gramme, and this concentration/

Table 2.

The concentrations of mercury in the tissues of rabbits after the intravenous injection of 5μM./kg. of mercuric chloride.

Time after injecting mercury	1 hour	6 hours	24 hours			5 days	14 days
Rabbit No.	367 ♂	371 ♂	361 ♀	365 ♂	369 ♂	363 ♂	359 ♂
Weight, kg.	1.50	1.55	2.55	1.80	1.33	1.87	2.25
	Micrognammes of mercury per gramme fresh weight of tissue.						
Plasma	0.97	<0.24	<0.04	<0.09	0.26	<0.11	<0.06
Erythrocytes	0.12	<0.33	0.66	<0.11	<0.30	<0.09	<0.09
Lungs	0.76	1.63	0.46	0.49	2.10	1.23	<0.04
Heart	<0.12	<0.52	<0.04	0.03	<0.20	-	-
Stomach	<0.02	<0.08	0.10	<0.02	0.30	0.04	0.29
Stomach contents	<0.03	0.20	0.41	0.85	1.10	0.97	0.18
Small intestine	0.08	1.18	0.29	0.51	0.61	0.40	0.35
" "contents	<0.01	1.04	0.34	0.53	1.09	0.16	<0.02
Caecum	-	-	0.65	0.43	0.67	0.88	-
Caecum contents	-	-	<0.04	0.89	<0.11	2.22	-
Colon	-	0.29	0.20	0.36	0.66	<0.09	<0.03
Colon contents	0.03	0.57	1.08	1.81	0.23	1.85	0.05
Kidneys	35.62	47.75	28.31	23.06	31.92	17.41	15.36
Liver	1.84	2.91	0.76	2.03	2.90	1.96	1.84
Bile	10.32	<2.01	<0.34	<1.71	-	<0.45	<0.81
Spleen	<0.80	<1.22	1.35	<0.65	<0.16	<1.01	<0.33
Skin	0.92	0.32	1.25	0.10	0.51	0.24	0.22
Brain	<0.05	-	<0.03	<0.05	<0.13	-	-
Bone marrow	2.58	<1.18	<0.24	<0.36	<0.51	<0.45	<0.13
Skull vault	-	-	<0.14	<0.31	<0.28	<0.21	<0.17
Epiphyses	0.50	0.42	0.11	<0.05	<0.09	<0.05	<0.03
Diaphyses	0.45	<0.23	0.13	<0.09	<0.20	0.32	<0.05
Skeletal muscle	<0.04	0.13	<0.03	<0.12	<0.07	0.04	<0.03
Total recovery	76.3%	81.2%	86.8%	82.4%	81.1%	80.3%	93.1%
Injection site	18.8%	26.2%	36.4%	32.4%	16.6%	3.3%	7.8%

Table 3.

The percentages of the dose of mercury in the tissues of rabbits after the intravenous injection of 5μM./kg. of mercuric chloride.

Time after injecting mercury	1 hour	6 hours	24 hours			5 days	14 days
Rabbit No.	367 ♂	371 ♂	361 ♀	365 ♂	369 ♂	363 ♂	359 ♂
Weight, kg.	1.50	1.55	2.55	1.80	1.33	1.87	2.25
Plasma	3.5	<1.7	<0.2	<0.3	1.4	<0.3	<0.3
Erythrocytes	0.4	<1.7	1.3	<0.3	<0.5	<0.3	<0.3
Lungs	0.4	0.5	0.2	0.2	1.0	0.6	<0.02
Heart	<0.03	<0.08	<0.01	0.01	<0.05	-	-
Stomach	<0.03	<0.08	0.1	<0.03	0.4	0.02	0.3
Stomach contents	<0.11	0.6	1.2	3.2	4.6	2.1	1.2
Small intestine	0.2	1.9	0.6	1.0	1.6	0.1	0.4
" " contents	<0.03	0.6	0.7	0.5	0.8	0.1	<0.02
Caecum	-	-	0.6	0.4	0.9	0.7	-
Caecum contents	-	-	<0.1	3.2	<0.6	5.1	-
Colon	-	0.1	0.1	0.2	0.4	<0.02	<0.02
Colon contents	<0.03	0.3	0.7	0.5	<0.1	0.5	0.04
Kidneys	22.6	20.6	19.8	13.2	22.3	18.4	10.2
Liver	7.3	10.6	2.9	6.3	9.2	4.3	5.3
Spleen	<0.03	<0.08	0.04	<0.03	<0.05	<0.06	<0.02
Skin	11.1	3.9	15.1	1.2	6.2	2.9	2.6
Brain	<0.03	-	<0.01	<0.03	<0.08	-	-
Bone Marrow	5.2	<2.3	<0.5	<0.7	<1.0	<0.9	<0.3
Bone	2.9	1.5	0.2	<0.5	<1.1	0.7	<0.2
Skeletal Muscle	<2.0	6.8	<1.7	6.6	<3.9	<2.4	<1.9
Excreted, urine	3.9	7.6	5.9	12.4	15.1	33.0	38.6
Excreted, faeces	-	-	1.0	1.1	0.6	8.5	26.7
Injection site	18.8	26.2	36.4	32.4	16.6	3.3	7.8
Total recovery	76.3%	81.2%	86.8%	82.4%	81.1%	80.3%	93.1%

concentration did not alter significantly during fourteen days after the injection of mercury. The next highest concentration usually occurred in the lungs, while heart, spleen and brain contained only very small quantites. A high concentration of mercury was found in bone marrow after one hour, but this fell below the detectable level later; similarly, the concentrations in bone decreased, and only very small amounts of mercury were found in this site after twenty-four hours. Skin usually held a few per cent of the dose, but skeletal muscle contained only small quantities. The amount of mercury in the gastro-intestinal tract rose during the first few hours, as excretion increased, then maintained a fairly steady concentration for a few days. Very small amounts occurred in the stomach, but the stomach contents usually showed a much higher concentration of mercury, perhaps because of the excretion of the metal in the saliva. After twenty-four hours, the small intestine, caecum, and colon contained small and similar concentrations, but again higher concentrations of mercury were generally found in the respective contents. A high concentration was found in/

in the bile after one hour, but thereafter the quantities of mercury in this fluid were below the level of detection.

About 4% of the dose was excreted in the urine within an hour, but this rate slowed considerably, and after twenty-four hours the urine contained 6-15% of the dose or 9-19% of the mercury absorbed. Only about two-fifths of the dose was excreted in the urine in fourteen days. About 1% of the dose was found in the faeces after twenty-four hours, but this rate of excretion increased, and nearly 27% was excreted in this way in fourteen days. Details of the excretion of mercury during the five-day and fourteen-day experiments are given in Tables 4 and 5.

The effect of administration of dimercaprol is shown in Tables 6 and 7. The doses of dimercaprol, and the times after the injection of mercury at which they were given, are set out in these tables. Generally, little change occurred in the qualitative aspect of the distributions, but considerable quantitative differences were found. The concentrations of mercury in most tissues tended to decrease,/

Table 4/

Fig. 1.

The concentrations of mercury found in samples of urine after the intravenous injection of 5 μM./kg. of mercuric chloride. Each arrow represents the injection of propylene glycol or of 25 mg./kg. of dimercaprol.

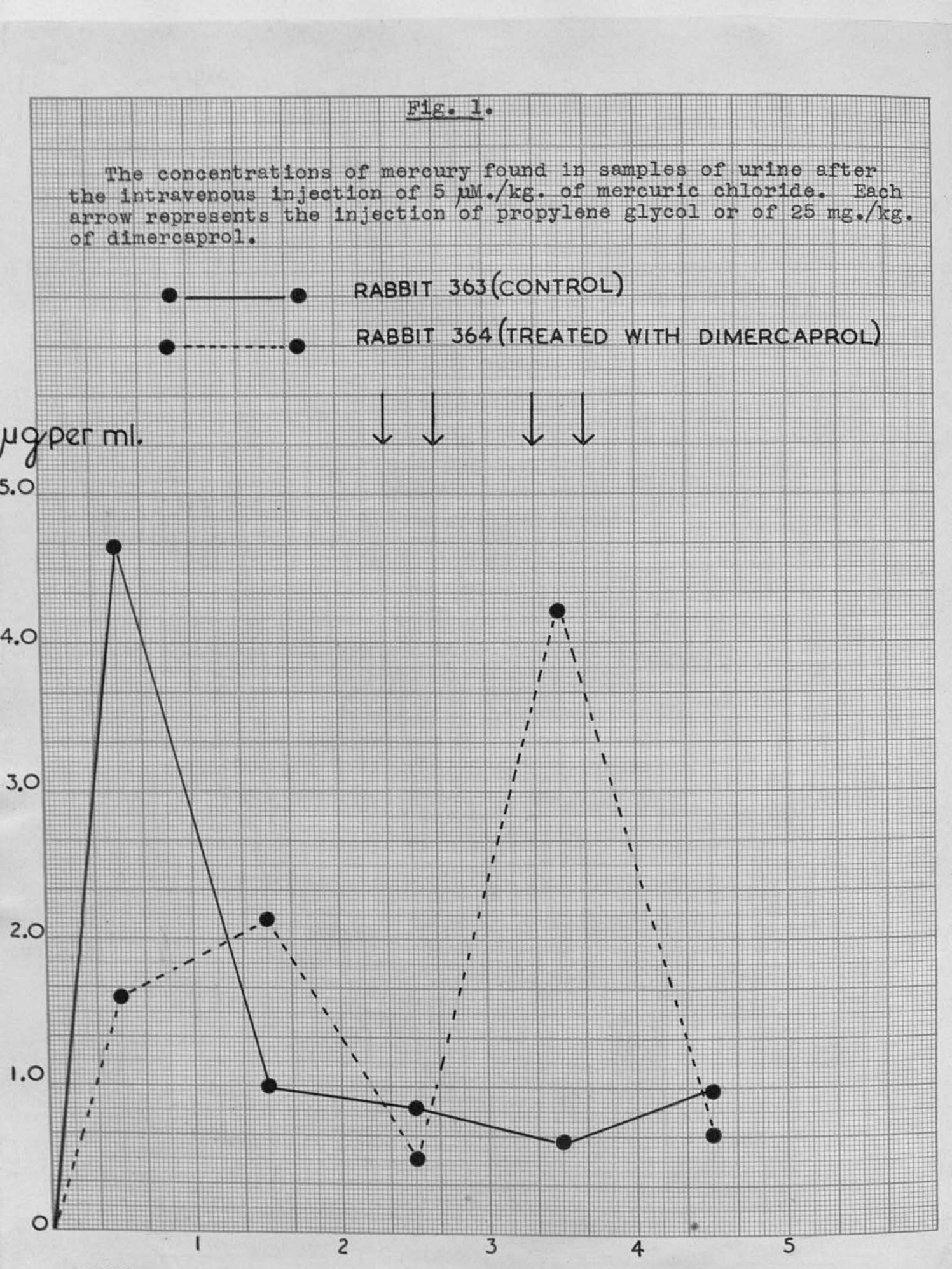


Table 4.

The excretion of mercury by rabbits during five days after the intravenous injection of 5μM./kg. of mercuric chloride. Excreta were collected at noon each day. Rabbit 364 was treated with 25mg./kg. of dimercaprol twice daily on days three and four.

Urine

		Rabbit 363		Rabbit 364		
	ml.	μg. Hg/ml.	% of dose	ml.	μg. Hg/ml.	% of dose
Days 0-1	90	4.64	22.3	66	1.62	6.0
" 1-2	58	0.97	3.0	60	2.10	7.1
" 2-3	52	0.84	2.3	24	0.46	2.0
" 3-4	56	0.60	1.8	64	4.20	15.0
" 4-5	70	0.96	3.6	110	0.66	4.1
Total mercury excreted.			33.0%			34.2%

Faeces

		Rabbit 363		Rabbit 364		
	g.	μg. Hg/g.	% of dose	g.	μg. Hg/g.	% of dose
Days 0-1	0	-	-	9.4	0.03	0.01
" 1-2	0	-	-	9.4	1.75	0.9
" 2-3	0	-	-	9.2	2.43	1.3
" 3-4	4.0	9.25	2.0	1.9	3.29	0.4
" 4-5	11.7	10.29	6.5	16.8	1.71	1.6
Total mercury excreted.			8.5%			4.2%

Fig.11.

The concentrations of mercury found in samples of urine after the intravenous injection of 5 μM./kg. of mercuric chloride. Each arrow represents the injection of propylene glycol or of 12.5 mg./kg of dimercaprol.

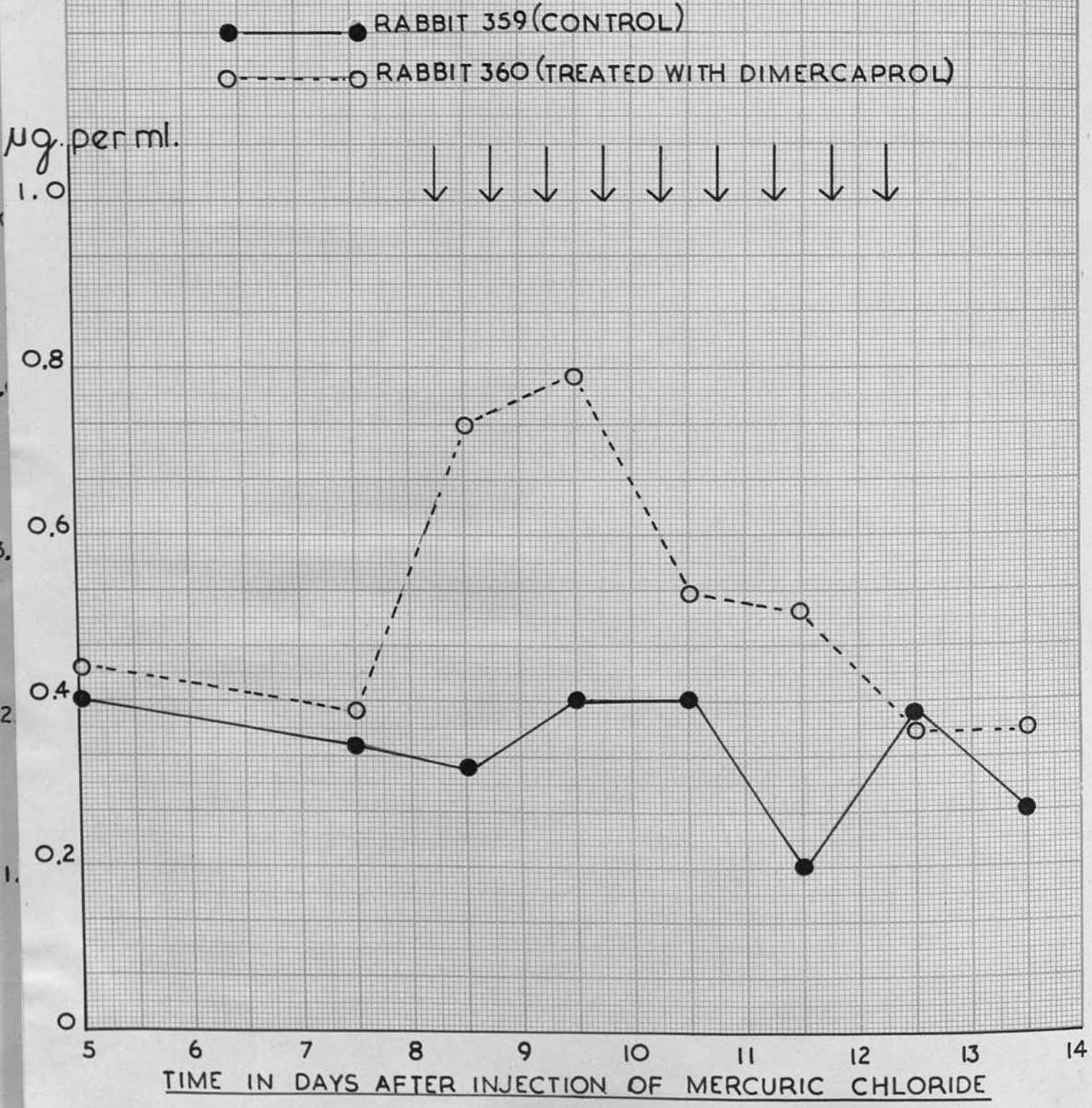


Table 5.

The excretion of mercury by rabbits during fourteen days after the intravenous injection of 5μM./kg. of mercuric chloride. Excreta were collected at noon, and therefore the period "Days 0-3" ends at noon on the third day after injection. Similarly, the period "Days 3-7" extends until noon on the seventh day. Rabbit 360 was treated with 12.5mg./kg. of dimercaprol twice daily on days 9-12, and once on day 13

Urine

	Rabbit 359			Rabbit 360		
	ml.	μg. Hg/ml.	% of dose	ml.	μg. Hg/ml.	% of dose
Days 0-3	215	1.86	17.8	70	4.99	22.7
" 3-7	665	0.40	11.9	270	0.44	7.8
" 7-8	62	0.35	1.0	92	0.39	2.3
" 8-9	190	0.32	2.7	80	0.73	3.8
" 9-10	33	0.40	0.6	46	0.79	2.4
" 10-11	76	0.40	1.3	59	0.53	2.0
" 11-12	78	0.21	0.7	42	0.51	1.4
" 12-13	26	0.39	0.5	32	0.37	0.8
" 13-14	174	0.27	2.1	105	0.37	2.5
Total mercury excreted.			38.6%			45.7%

Faeces

	Rabbit 359			Rabbit 360		
	g.	μg. Hg/g.	% of dose	g.	μg. Hg/g.	% of dose
Days 0-3	5.5	1.78	0.4	10.0	2.02	1.3
" 3-7	31.0	9.47	13.0	77.0	3.34	16.7
" 7-8	0	-	-	0	-	-
" 8-9	10.5	9.89	4.6	8.0	2.18	1.1
" 9-10	-	-	-	7.7	0.99	0.5
" 10-11	5.0	6.10	1.4	13.4	1.56	1.4
" 11-12	8.4	4.58	1.7	12.3	0.96	0.8
" 12-13	1.7	3.63	0.3	13.9	0.94	0.7
" 13-14	42.7	2.76	5.3	20.3	0.32	0.4
Total mercury excreted.			26.7%			22.9%

decrease, and this effect was most marked in those tissues containing the highest concentrations in untreated animals. The amounts of mercury in the kidneys fell profoundly. The concentration of mercury in the liver were generally, but not significantly, smaller than those of the untreated animals. The quantities of mercury in the gastro-intestinal tract tended to decrease, but no definite effect on the excretion of mercury in the faeces was observed. Smaller amounts of mercury were usually found in skin, but the decrease was not significant. The loss of mercury from these tissues was paralleled, and accounted for, by a greatly increased excretion of mercury in the urine, and possibly by an increase in the amounts in skeletal muscle in some cases.

These actions of dimercaprol were found, though on a smaller scale, even when treatment was delayed until nine days after the administration of mercury. The two rabbits killed after five days showed similar amounts of mercury in the urine, but it should be noted that the rabbit (No.364), treated with dimercaprol, retained about 30% of the dose at the site of injection.

The/

Table 6.

The concentrations of mercury in the tissues of rabbits after the intravenous injection of 5μM./kg. of mercuric chloride, and subsequent treatment with dimercaprol.

Time after injecting mercury	1 hr.	6 hrs.	24 hours		5-days	14-days
Treatment with dimercaprol.	50mg/kg immediately	50mg/kg at 1 hr 12.5mg/kg. at 5 hours	50mg./kg at 1-hr. 12.5mg/kg. at 5 hours.	50mg./kg at 19-hrs. and 12.5 mg/kg at 23-hrs	25mg/kg twice daily on days 3 and 4.	12.5mg/kg twice daily on days 9-12 and once on day 13.
Rabbit No.	368 ♂	372 ♀	370 ♀	362 ♂	364 ♂	360 ♂
Weight, kg.	1.40	1.86	2.05	1.72	1.78	1.54
	Micrograms of mercury per gramme fresh weight of tissue.					
Plasma	1.66	<0.19	<0.10	<0.07	<0.08	<0.06
Erythrocytes	2.82	1.24	2.42	<0.07	<0.10	<0.11
Lungs	2.41	<0.18	<0.08	<0.04	<0.06	<0.05
Heart	0.34	<0.27	<0.14	<0.06	-	-
Stomach	0.71	0.20	<0.03	0.16	<0.03	0.06
Stomach contents	0.26	<0.11	0.49	<0.01	0.22	<0.02
Small intestine	1.43	0.95	0.12	0.24	0.21	0.38
" " contents	2.65	<0.12	0.96	1.02	<0.04	0.13
Caecum	-	-	0.36	<0.01	<0.03	0.01
Caecum contents	-	-	<0.12	0.46	0.59	0.13
Colon	0.64	0.58	<0.07	<0.02	<0.06	<0.07
Colon contents	<0.05	0.61	<0.06	0.26	1.00	0.16
Kidneys	8.79	1.83	6.65	3.30	1.78	1.95
Liver	1.11	1.19	1.23	0.41	0.64	0.50
Bile	2.30	<2.22	1.51	<1.36	<0.85	<1.33
Spleen	<0.81	<1.64	2.99	<0.64	<0.83	<0.40
Skin	0.52	<0.29	<0.15	<0.04	0.33	<0.09
Brain	<0.07	-	<0.09	<0.03	<0.06	-
Bone marrow	<0.30	<0.63	<0.45	<0.35	<0.32	<0.32
Skull vault	-	-	<0.46	<0.21	<0.27	<0.30
Epiphyses	<0.05	<0.18	<0.09	0.03	<0.04	<0.06
Diaphyses	<0.09	<0.22	<0.12	<0.06	0.37	<0.08
Skeletal muscle	0.32	0.50	<0.07	0.08	0.11	0.07
Total recovery	82.0%	90.0%	86.7%	81.0%	87.3%	76.9%
Injection site	11.7%	19.2%	10.6%	32.4%	36.2%	29.6%
						89.7%
						5.6%

Table 7.

The percentages of the dose of mercury in the tissues of rabbits after the intravenous injection of 5μM./kg. of mercuric chloride, and subsequent treatment with dimercaprol.

Time after injecting mercury	1-hr.	6-hrs.	24 hours			5-days	14-days
Treatment with dimercaprol.	50mg/kg immediately.	50mg/kg at 1hr. 12.5mg/kg. at 5-hrs.	50mg/kg at 1-hr 12.5mg/kg at 5 hours.	50mg/kg at 19-hrs. and 12.5 mg/kg at 23 hours.		25mg/kg twice daily on days 3 and 4.	12.5mg/kg twice daily on days 9-12 and once on day 13.
Rabbit No.	368 ♂	372 ♀	370 ♀	362 ♂	366 ♂	364 ♂	360 ♂
Weight, kg.	1.40	1.86	2.05	1.72	2.37	1.78	1.54
Plasma	7.1	<0.9	<0.5	<0.3	<0.5	<0.3	<0.3
Erythrocytes	7.8	2.8	5.8	<0.3	<0.5	<0.3	<0.3
Lungs	0.9	<0.07	<0.03	<0.02	0.2	<0.02	<0.02
Heart	0.07	<0.07	<0.03	<0.02	<0.02	-	-
Stomach	0.8	0.2	<0.03	0.2	<0.02	<0.02	0.06
Stomach contents	1.4	<0.4	2.8	<0.05	0.7	0.9	<0.2
Small intestine	2.9	1.6	0.2	0.6	0.2	0.3	0.5
Small " contents	4.1	<0.07	0.5	0.8	<0.02	0.2	0.3
Caecum	-	-	<0.4	<0.02	<0.02	0.01	-
Caecum contents	-	-	<0.6	2.0	1.7	0.6	-
Colon	0.3	0.3	<0.03	<0.02	<0.02	<0.02	<0.02
Colon contents	<0.03	0.2	<0.06	0.3	0.06	0.1	0.06
Kidneys	5.8	1.4	4.6	2.5	1.0	1.5	1.2
Liver	3.3	3.9	4.7	2.0	2.1	1.4	3.3
Spleen	<0.03	<0.07	0.3	<0.04	<0.02	<0.06	<0.03
Skin	6.3	<3.4	<1.8	<0.5	4.0	<0.11	0.7
Brain	<0.03	-	<0.03	<0.02	<0.02	-	-
Bone Marrow	0.6	<1.2	<0.9	<0.7	<0.62	<0.6	<0.4
Bone	<0.4	<1.2	<0.9	0.05	0.8	<0.6	<0.3
Skeletal muscle	16.7	26.3	<3.9	4.4	5.6	3.9	9.4
Excreted, urine	12.8	34.1	56.1	32.1	33.8	34.2	45.7
Excreted, faeces	-	-	0.7	3.7	0.9	4.2	22.9
Injection site	11.7	19.2	10.6	32.4	36.2	29.6	5.6
Total recovery	82.0%	90.0%	86.7%	81.0%	87.3%	76.9%	89.7%

The amounts of mercury excreted in both urine and faeces during the five and fourteen days experiments are shown in Tables 4 and 5. When dimercaprol was administered three days after the mercury, an increase occurred in the concentration of mercury in the urine, as shown in Fig.1. Treatment begun nine days after the mercury had a similar effect, but this disappeared rapidly, in spite of continued treatment, (Fig.2).

The results of treatment with dimercaprol glucoside are presented in Tables 8 and 9. Considerable differences from the actions of dimercaprol were found. The mercury content of the plasma was consistently raised, and in the cases of rabbits No.400 and 402, the content of the erythrocytes was also increased. Large increases in the concentration of mercury in the bile occurred, but the amounts of mercury contained in the small intestine, colon, and their contents, were generally similar to those found in untreated animals. No definite change in the concentration of mercury in the liver was observed, but the mercury content of the kidneys was diminished/

Table 8

The concentrations of mercury in the tissues of rabbits after the intravenous administration of mercuric chloride, and subsequent treatment with dimercaprol glucoside.

Time after injecting mercury	1 hour	24 hours					
Dose of mercury	5μM./kg.	5μM/kg.				3.7μM./kg.	
Treatment with dimercaprol glucoside.	175 mg./kg. immediately.	175mg./kg. at 1 hour and 55mg./kg. at 5hrs.		175mg./kg. at 19 hours and 55mg./kg. at 23 hours.		175mg./kg. at 1 hour and 55mg./kg. at 5 hours.	
Rabbit No.	410 ♂	400 ♀	402 ♀	406 ♂	401 ♀	404 ♀	411 ♂
Weight, kg.	1.85	1.75	1.75	1.75	1.72	1.69	1.67
	Microgrammes of mercury per gramme fresh weight of tissue.						
Plasma	3.67	1.64	1.25	2.02	1.07	0.67	1.37
Erythrocytes	<0.23	2.57	5.35	<0.07	0.90	0.11	<0.16
Lungs	1.66	0.87	1.63	0.53	1.30	0.68	1.49
Small intestine	-	<0.01	1.12	-	0.69	0.14	1.52
" " contents	-	1.10	1.25	-	1.37	0.04	0.19
Colon	-	0.66	1.14	0.75	0.80	0.61	1.46
" contents	-	1.84	7.30	0.55	-	0.14	-
Kidneys	3.32	4.76	6.25	1.93	4.82	2.22	1.01
Liver	4.72	2.27	2.48	1.58	1.46	0.66	1.91
Bile	3.59	6.29	2.56	6.19	14.62	7.70	7.10
Skin	1.42	0.66	1.80	0.47	-	<0.08	<0.18
Skeletal muscle	<0.07	0.08	<0.02	0.05	0.09	<0.02	0.10
Total recovery	71.5%	71.5%	83.0%	73.8%	71.6%	77.4%	85.6%
Injection site	8.1%	0.5%	1.8%	15.3%	23.8%	0.6%	0.04%

Table 9.

The percentages of the dose of mercury in the tissues of rabbits after the intravenous administration of mercuric chloride, and subsequent treatment with dimercaprol glucoside.

Time after injecting mercuric chloride.	1 hour	24 hours					
Dose of mercuric chloride.	5μM./kg.	5μM./kg.				3.7μM./kg	
Treatment with dimercaprol glucoside.	175 mg./kg. immediately.	175mg./kg. at 1 hour and 55mg./kg. at 5hrs.		175mg./kg. at 19 hours and 55mg./kg. at 23 hours.		175mg./kg. at 1 hour and 55mg./kg. at 5 hours.	
Rabbit No. Weight, kg.	410 ♂ 1.85	400 ♀ 1.75	402 ♀ 1.75	406 ♀ 1.75	401 ♀ 1.72	404 ♀ 1.69	411 ♂ 1.67
Plasma	12.8	6.7	4.8	8.5	4.5	4.3	8.3
Erythrocytes	< 0.8	7.5	16.7	< 0.2	2.5	0.3	< 0.5
Lungs	0.7	0.3	0.5	0.2	0.6	0.5	0.9
Small intestine	-	< 0.02	1.9	-	1.5	0.4	5.1
" " contents	-	0.5	0.4	-	0.4	0.04	0.2
Colon	-	0.3	0.5	0.4	0.4	0.3	1.1
Colon contents	-	1.3	1.1	0.1	-	0.1	-
Kidneys	2.2	2.8	4.7	1.5	3.9	2.0	0.9
Liver	13.3	8.3	7.3	6.1	5.6	5.4	8.8
Skin	17.0	11.4	21.6	8.8	-	< 1.1	< 2.9
Skeletal muscle	< 3.9	4.9	< 1.3	3.5	6.9	< 1.9	7.0
Excreted, urine	17.4	24.5	20.2	29.2	20.6	62.8	45.3
Excreted, faeces	-	2.5	1.5	0.2	0.9	0.7	8.0
Injection site	8.1	0.5	1.8	15.3	23.8	0.6	< 0.04
Total recovery	71.5%	71.5%	83.0%	73.8%	71.6%	77.4%	85.6%

diminished to an extent similar to that occurring in dimercaprol-treated animals. The quantities of mercury in skin tended to increase, but the difference was not significant.

The urinary excretion of mercury, while greater than that of the control animals, was smaller than that obtained after treatment with dimercaprol. Calculating the quantities excreted after twenty-four hours as the percentages of the mercury absorbed, the mean for the control animals was 15%, for the animals treated with dimercaprol 55%, and for those treated with the glucoside 29%; all these differences were significant ($P < 0.01$). This comparatively small increase in the excretion of mercury after treatment with dimercaprol glucoside was surprising, and the effect of reducing the dose of mercuric chloride was investigated. Only a limited decrease in the dose was possible owing to the increased error which resulted from lowering the counts obtained from samples. Accordingly, the dose of mercuric chloride was reduced to 3.7μM./kg. (0.75mg. Hg./kg.), and the animals were treated with dimercaprol/

dimercaprol glucoside as previously (Tables 8, 9, and 10). In these cases, smaller fractions of the dose were generally found in the erythrocytes, and larger fractions in the urine, than in the cases of animals receiving 5μM./kg. of mercuric chloride. However, the difference between the actual weights of mercury excreted by the two groups was not significant.

Rabbit 409 (Table 10) was injected with 3.7μM./kg. of mercuric chloride, and was not treated; the urinary excretion of mercury amounted to 23% of the dose, whereas that of the control animals receiving 5μM./kg. was 15%. This increased excretion was probably mainly due to the smaller quantity of mercury remaining at the injection site, and not to the reduction in the dose. One rabbit (No.407) was given dimercaprol glucoside in three equal doses, instead of one large dose followed by a smaller one. There was no alteration in the quantity of mercury excreted. A further experiment (rabbit No.405) confirmed the excretion figures, after dimercaprol treatment, obtained previously.

The/

Table 10.

Additional estimations of the percentages of the dose of mercury in urine and blood 24-hours after the intravenous administration of mercuric chloride in rabbits.

Dose of mercuric chloride.	3.7μM./kg.			5μM./kg.			
Treatment	Nil	Dimercaprol glucoside 175mg./kg. at 1hr and 55mg./kg. at 5hrs.		Dimercaprol glucoside 175mg./kg. at 1hr. and 55mg./kg. at 5hrs.		Dimercaprol glucoside 75mg./kg. at 1, 4 and 7hrs.	Dimercaprol 50mg./kg. at 1hr. and 12.5mg./kg. at 5hrs.
Rabbit No. Weight, kg.	409 ♂ 1.57	408 ♂ 1.70	413 ♂ 1.78	403 ♂ 1.97	412 ♂ 1.87	407 ♀ 1.79	405 ♀ 1.94
Injection site	0.3	0.4	3.5	2.1	5.6	0.2	0.9
Plasma	1.9	-	-	-	-	7.3	<0.3
Erythrocytes	<0.3	-	-	-	-	<0.1	<0.5
Urine	23.1	53.2	48.3	32.5	42.2	34.3	56.9

FIG. 3a.

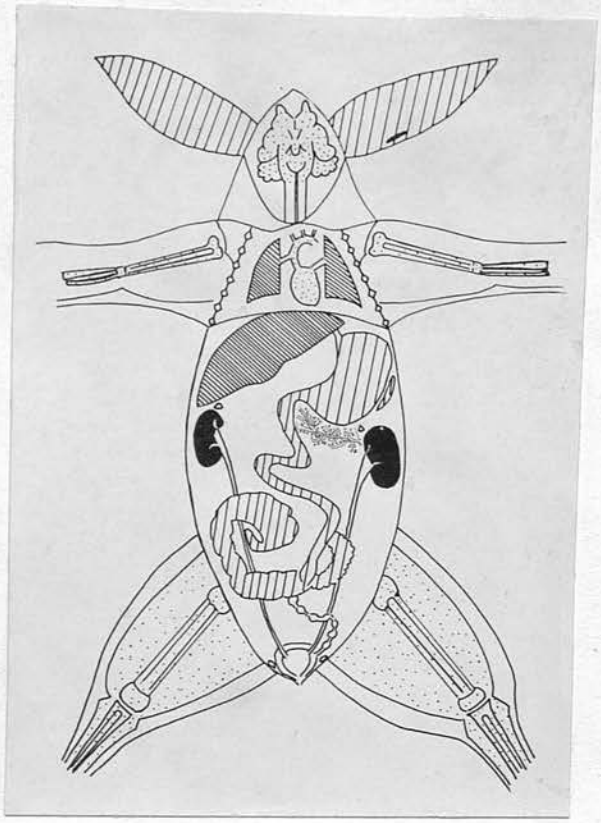


FIG. 3b.

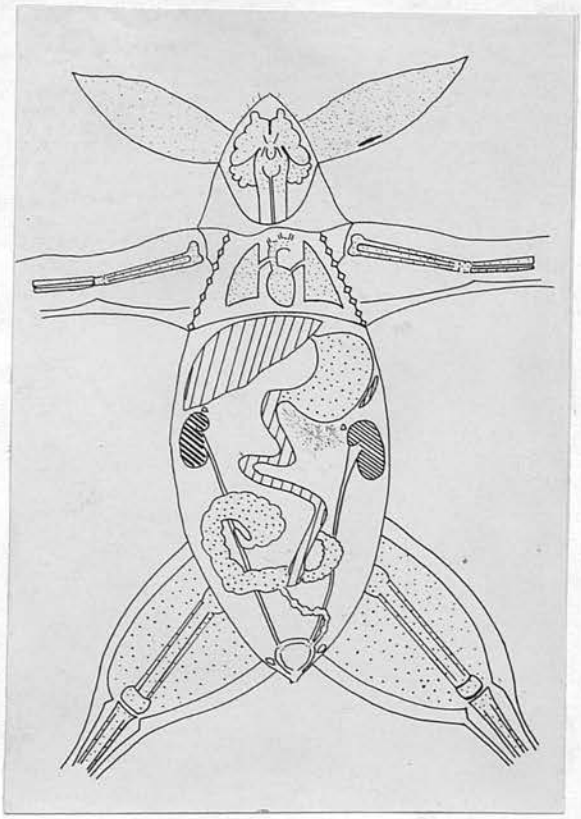


FIG. 3c.

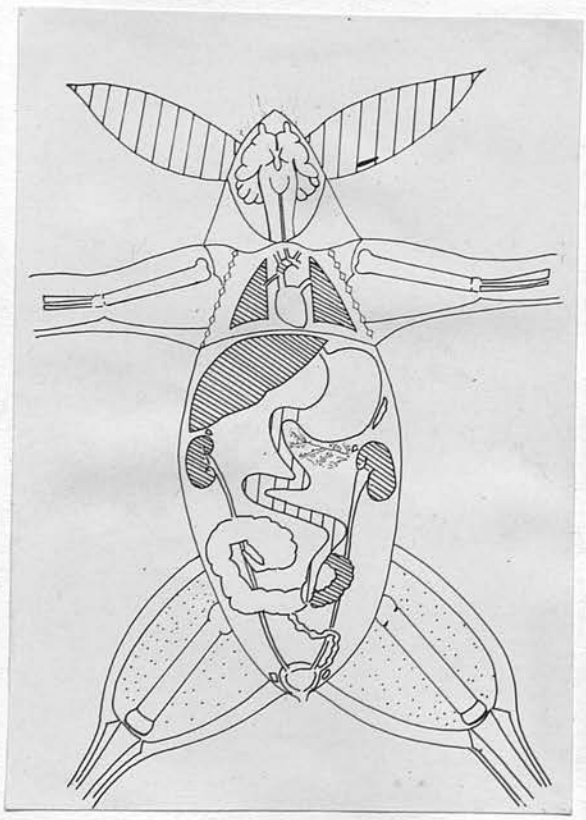


Fig. 3 (a). The mean concentrations of mercury in various tissues and organs twenty--four hours after the intravenous administration of 5 μM./kg. of mercuric chloride.

Fig. 3 (b). The mean concentrations of mercury in various tissues and organs twenty--four hours after the intravenous administration of 5 μM/kg. of mercuric chloride and subsequent treatment with dimercaprol.

Fig. 3 (c). The mean concentrations of mercury in various tissues and organs twenty--four hours after the intravenous administration of 5 μM./kg. of mercuric chloride, and subsequent treatment with dimercaprol glucoside.

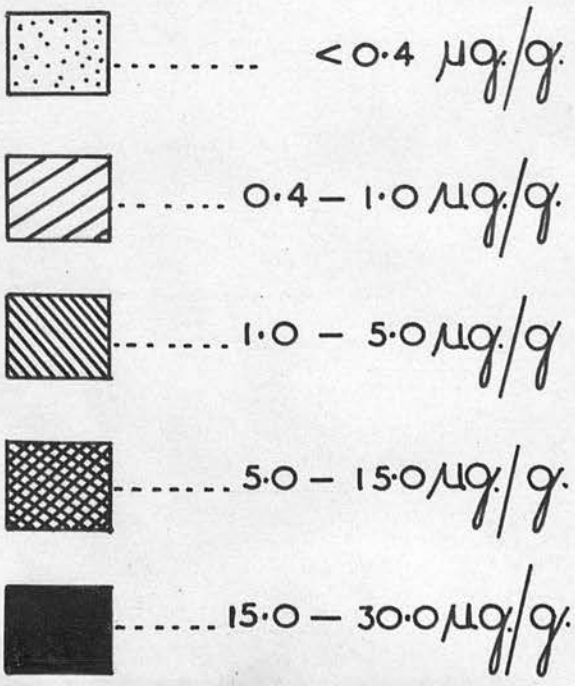
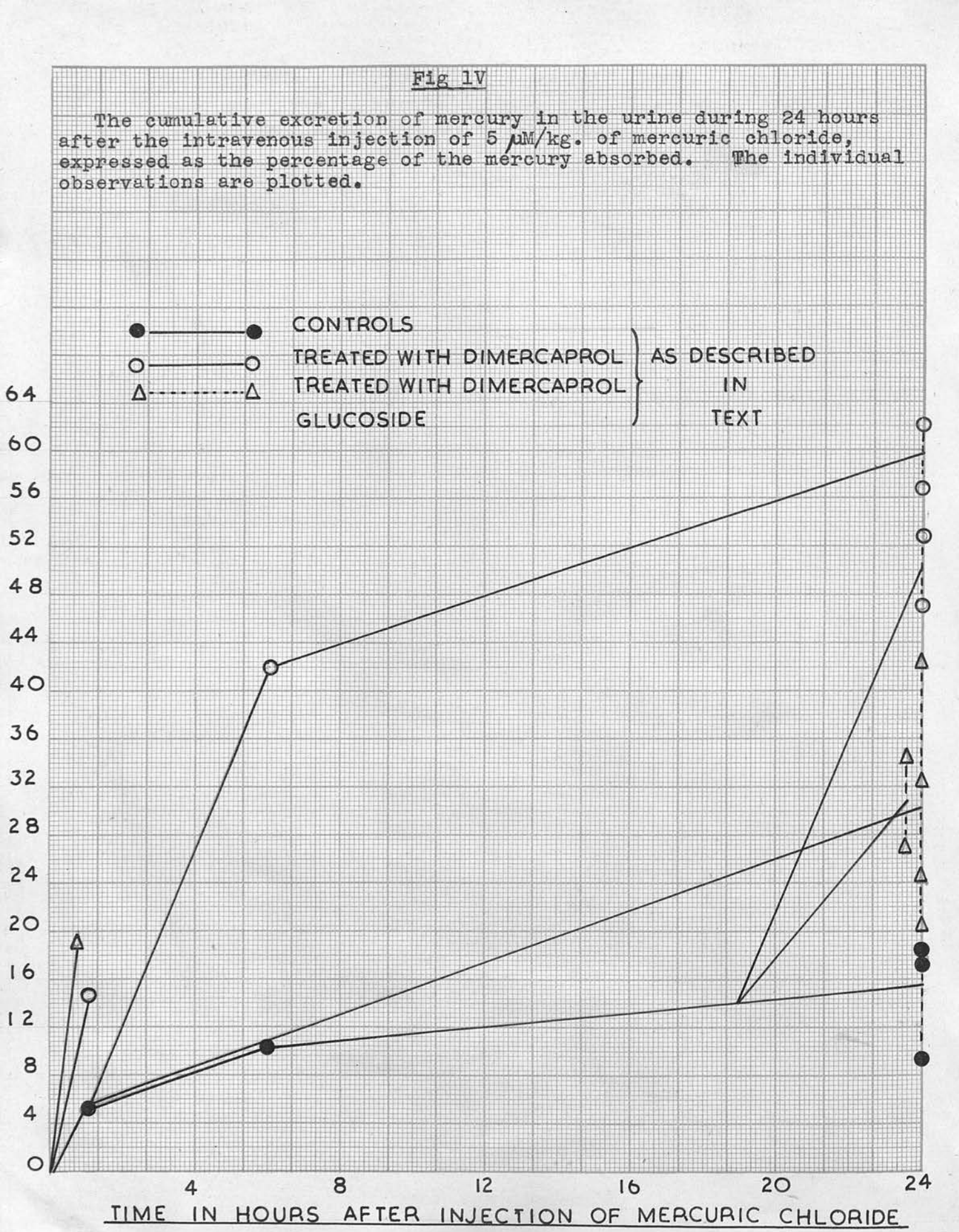


Fig 1V

The cumulative excretion of mercury in the urine during 24 hours after the intravenous injection of 5 μM/kg. of mercuric chloride, expressed as the percentage of the mercury absorbed. The individual observations are plotted.



The main changes in the tissue distribution of mercury, after treatment with dimercaprol or dimercaprol glucoside, are illustrated in Fig.3. The concentrations of mercury found in the various tissues are depicted, and were calculated as the average of the figures obtained in those animals killed at the end of the twenty-four hour period. A summary of the mean percentages of the dose found in various tissues is given in Table 11.

The cumulative excretion of mercury in the urine during twenty-four hours without and with treatment by dimercaprol or dimercaprol glucoside, is illustrated in Fig.4. The percentages of the absorbed mercury have been calculated, and the ranges covered by the individual observations at twenty-four hours are shown.

In these diagrams, the results tend to be oversimplified, but it is considered that this is justified by the more immediate comprehensibility of the date.

Table 11/

Table 11.

Summary table of the distribution of mercury in rabbits, without and with treatment with dimercaprol or dimercaprol glucoside. The figures given are the mean percentages of the dose found in each group of tissues 24-hours after the intravenous administration of 5μM./kg. of mercuric chloride. Details of treatment are as shown in Tables 3, 7, and 9.

Treatment	None	Dimercaprol	Dimercaprol glucoside
No. of rabbits	3	3	4
Blood	0.9	1.9	12.8
Small intestine) " " contents) Colon) Colon contents) Faeces)	3.3	3.0	4.4
Kidneys	18.4	2.7	3.2
Liver	6.1	2.9	6.8
Skin) Skeletal muscle)	9.7	4.7	17.7
Urine	11.1	40.7	27.2

PART II: Gold.

In view of the short half-life of Au^{198}, of the inconveniences of supply, and the satisfactory results repeatedly obtained with Pb^{212}, the technique of ashing and estimation used for the latter was applied, without preliminary checking, to studies on the former. Consistently poor recoveries indicated that considerable losses of gold occurred during this procedure. Possibly volatilization of the gold took place through overheating, for Block and Buchanan (1940) emphasize that gentle heating is necessary during the ashing of tissues containing gold. The pertinent recovery experiments with mercury (vide p.24) had not been carried out at the time of this work, and the short half-life of Au^{198} precluded extensive investigations into the cause of the losses.

Nevertheless, the error of the duplicate estimations was only $\pm$ 8%, which indicates that approximately similar proportions of gold were lost from each sample. Thus, the true relative amounts of gold in different tissues are probably about the same/

same as found in these experiments, but the actual concentrations of gold are likely to be higher. Again, urine and bile samples were not ashed, and hence were not subject to this loss of gold.

Results are presented showing the distribution and excretion of gold in eight rabbits, all of which received a single dose of 0.01mM./kg. (1.97mg. Au/kg.) of gold chloride intravenously, and were killed twenty-four hours or five days after the administration of gold. Four of the rabbits received treatment with dimercaprol, as detailed in Tables 12 and 13. No ill effects were noted, except in the case of rabbit No.387 in which mild convulsions occurred after the first injection of dimercaprol.

The concentrations of gold in microgrammes per gramme of tissue and the percentages of the dose in various organs and tissues are presented in Tables 12 and 13. After twenty-four hours, a large proportion of the gold was still circulating in the blood, mainly in the plasma, of the control animals. Very high concentrations were found in the spleen. The kidneys contained the next highest concentration, about 8-12µg. of gold per gramme, while the liver held/

held only 2-4µg. per gramme and accounted for about 3% of the dose. The bile contained 2-5µg. of gold per ml. and, on the limited information obtained, the gastro-intestinal tract and its contents held an average of about 1µg. per gramme. Bone marrow contained 4-8µg. of gold per gramme, and accounted for 4-8% of the dose; the concentration in bone was lower, the epiphyses containing 1-2µg. and the diaphyses roughly 1µg. per gramme. The very high concentration of gold in the sample of skull vault from rabbit No. 201 seems anomalous. The lungs contained 1-4µg. of gold per gramme, while heart and brain held only very small quantities. The concentrations in skin and skeletal muscle were also low, but accounted for 10-20% of the dose.

Only 3-8% of the gold administered was excreted in the urine in twenty-four hours, and less than 1% in the faeces. After five days, urinary excretion still accounted for only 8% of the dose, but 10% was found in the faeces over that period. Details of the excretion of gold in this experiment are given in Table 14.

Table 12/

Table 12.

The concentrations of gold in the tissues of rabbits after the intravenous injection of 0.01mM./kg. of gold chloride, without and with treatment with dimercaprol.

Time after injecting gold.	24 hours						5 days	
Treatment with dimercaprol.	Nil			50mg./kg. at 19 hrs and 12.5mg./kg. at 23 hours.			Nil	25m.g/kg. on days 2, 3 and 4.
Rabbit No.	201 ♂	200 ♀	202 ♀	210 ♀	211 ♂	212 ♂	203 ♀	213 ♀
Weight, kg.	2.10	2.25	2.00	1.95	2.15	1.95	2.15	2.15
	Microgrammes of gold per gramme fresh weight of tissue.							
Plasma	7.11	3.22	6.33	2.47	1.34	2.47	1.01	0.75
Erythrocytes	1.28	1.36	1.30	1.04	0.36	0.79	0.15	<0.05
Heart	0.37	-	-	0.54	-	-	-	-
Lungs	3.19	1.32	4.13	0.75	13.54	3.13	3.77	1.01
Stomach	1.13	-	-	0.70	-	-	-	-
Stomach contents	0.66	-	-	0.55	-	-	-	-
Small intestine	1.41	0.12	-	0.62	0.45	-	-	-
" " contents	2.67	1.94	-	0.95	0.14	-	-	-
Colon	1.00	0.95	-	0.70	0.26	-	-	-
Colon contents	1.63	0.46	-	4.00	0.68	-	-	-
Kidneys	9.84	11.82	7.79	11.14	12.84	7.67	10.42	6.31
Liver	2.89	1.93	4.50	1.26	3.31	3.57	4.31	2.63
Bile	5.43	2.18	3.06	-	8.28	9.26	0.30	0.61
Spleen	37.00	22.03	11.85	35.29	12.67	21.17	5.61	12.18
Skin	0.71	0.86	-	1.36	0.36	-	-	-
Bone marrow	3.99	8.20	4.69	4.27	4.42	4.01	1.25	4.06
Epiphyses	1.81	1.52	2.65	-	18.92	0.31	0.39	1.72
Diaphyses	0.26	0.68	1.71	0.55	0.68	0.66	0.23	0.43
Skull vault	11.81	-	1.86	3.02	-	0.85	0.97	0.51
Skeletal muscle	0.20	0.51	-	0.38	0.67	-	0.21	0.05
Brain	0.05	0.17	-	0.26	0.04	-	-	-

Table 13.

The percentages of the dose of gold in the tissues of rabbits after the intravenous injection of 0.01mM./kg. of gold chloride, without and with treatment with dimercaprol

Time after injecting gold.	24-hours						5-days	
Treatment with dimercaprol	Nil			50mg/kg at 19hrs and 12.5mg/kg at 23-hrs.			Nil	25mg./kg. on days 2, 3 and 4.
Rabbit No.	201♂	200♀	202♀	210♀	211♂	212♂	203♀	213♀
Weight, kg.	2.10	2.25	2.00	1.95	2.15	1.95	2.15	2.15
Plasma	13.2	8.0	10.5	4.8	2.1	4.8	2.4	1.5
Erythrocytes	1.7	1.6	1.3	0.9	0.7	1.4	0.2	<0.05
Heart	0.04	-	-	0.05	-	-	-	-
Lungs	0.7	0.2	0.7	0.1	2.7	0.6	0.8	0.2
Stomach	5.6	-	-	2.5	-	-	-	-
Stomach contents		-	-		-	-	-	-
Small intestine		1.1	-		0.9	-	-	-
Small " contents			-			-	-	-
Colon			-			-	-	-
Colon contents			-			-	-	-
Kidneys	2.5	1.7	1.8	2.7	3.7	2.1	2.3	1.7
Liver	3.2	2.9	3.5	1.9	4.4	4.1	5.9	3.1
Spleen	0.2	0.2	0.2	0.5	0.2	0.2	0.1	0.3
Skin	5.2	7.2	-	6.6	2.3	-	-	-
Bone marrow	3.7	8.6	3.5	3.5	4.5	4.1	1.2	4.0
Bone	4.5	4.5	5.0	7.3	26.7	1.1	1.2	5.2
Skeletal muscle	4.7	13.9	-	8.0	18.1	-	5.4	1.4
Brain	0.01	0.03	-	0.04	0.01	-	-	-
Excreted, urine	2.9	3.9	5.3	6.6	8.3	8.9	7.7	14.8
Excreted, faeces	0.7	0.5	-	0.3	0.4	-	10.4	8.2
Injection site	0.8	0.3	0.2	9.2	2.9	1.0	10.3	0.4
Total recovery	49.6%	54.6%	32.0%	55.0%	77.9%	28.3%	47.9%	40.8%

Much smaller concentrations of gold were detected in the spleen, blood, bile, bone marrow and bone after the five-day period. The concentrations in the liver, kidneys, lungs and skeletal muscle remained fairly constant. It should be noted that 10% of the dose was retained at the injection site in this rabbit (No.203).

The results of treatment with dimercaprol are also presented in Tables 12 and 13. The most significant effect was a decrease in the concentrations of gold in the blood after twenty-four hours, the amounts in both plasma and erythrocytes being approximately halved. The concentrations of gold in the bile increased but there was no observable effect on the gold content of other tissues. No explanation can be offered for the very high concentration of gold found in the epiphyses of rabbit No.211.

The excretion of gold in the urine was approximately doubled after dimercaprol treatment, but there was no effect on the faecal excretion.

Even when treatment was delayed until two days after the administration of gold, dimercaprol still caused/

caused an increased urinary excretion of the metal. Smaller amounts of gold were found in the blood, liver, kidneys, and skeletal muscle of the treated rabbit than in the control animal, and larger quantities occurred in bone and bone marrow. As some of these effects are at variance with those found after the twenty-four hour period, and only one set of results is available for the five-day period, the differences cannot be definitely ascribed to the action of dimercaprol. The effect of treatment on the excretion of gold in the urine in the five-day experiment is illustrated in Fig.5, and details of the urinary and faecal excretion are given in Table 14.

To ensure that no large amounts of gold occurred in sites which were not usually sampled, the gold contents of various additional tissues in rabbits Nos. 202 and 212 were estimated. The results are shown in Table 15. None of these tissues contained any notable concentrations of gold, and a reduction in the concentrations appeared to occur in most of them after treatment with dimercaprol.

Table 14/

Fig. V.

The concentrations of gold found in samples of urine after the intravenous injection of 0.01 mM./kg. of gold chloride. Each arrow represents the injection of propylene glycol or of 25 mg./kg. of dimercaprol.

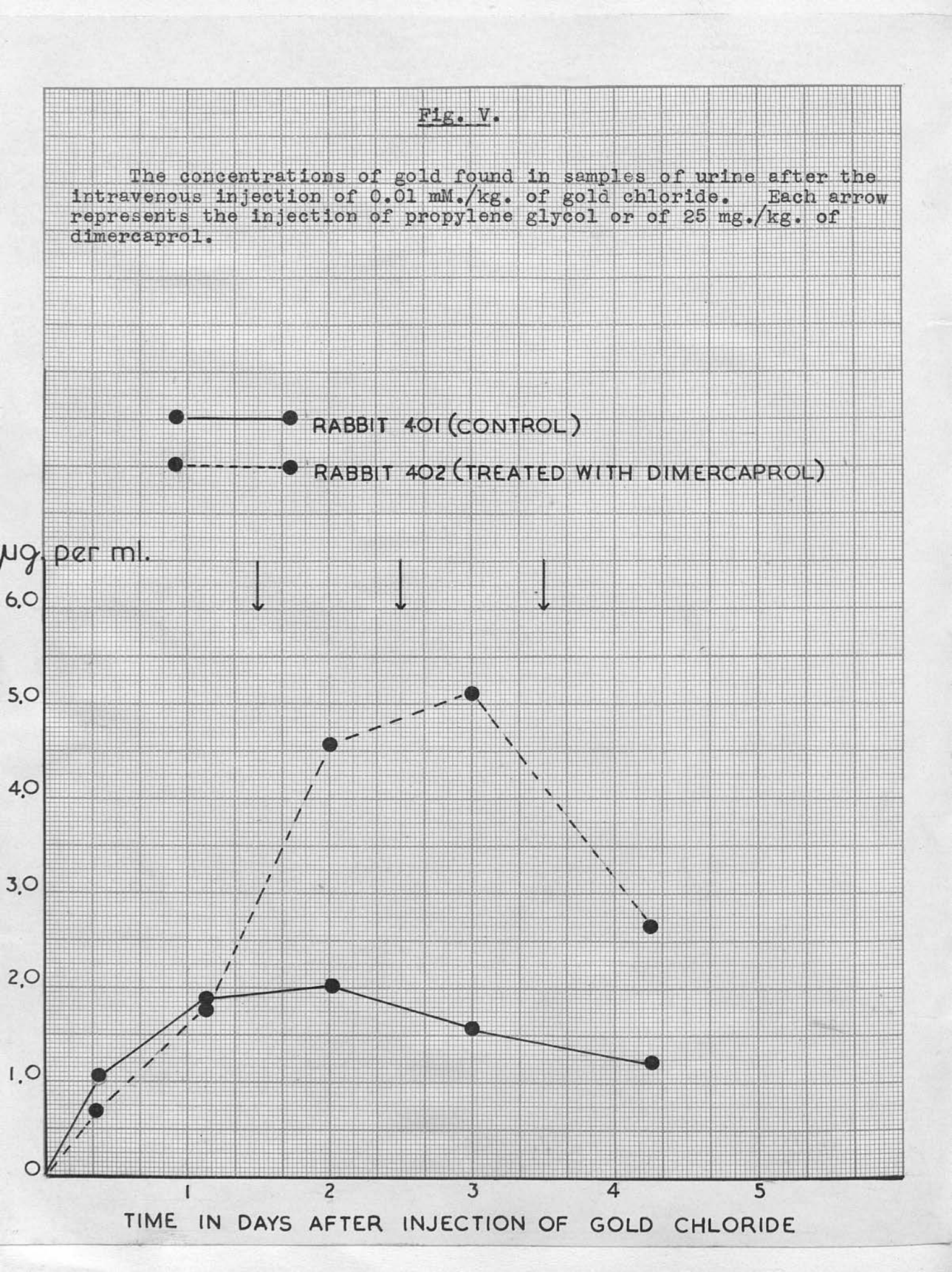


Table 14.

The excretion of gold by rabbits during five days after the intravenous injection of 0.01mM./kg. of gold chloride. Rabbit 213 was treated with 25mg./kg. of dimercaprol daily on days 2, 3, and 4.

Urine

	Rabbit 203			Rabbit 213		
	ml.	µg. Au/ml.	% of dose	ml.	µg. Au/ml.	% of dose
0-18 hours	22	1.96	1.0	16	1.84	0.7
18-36 hours	31	2.50	1.8	27	2.79	1.8
36-60 hours	52	1.72	2.1	75	2.58	4.6
60-84 hours	52	1.31	1.6	87	2.48	5.1
84-120 "	58	0.88	1.2	85	1.41	2.6
Total gold excreted.			7.7%			14.8%

Faeces

	Rabbit 203			Rabbit 213		
	g.	µg. Au/g.	% of dose	g.	µg. Au/g.	% of dose
0-18 hours	115	0.37	1.0	59	0.13	0.2
18-36 hours	104	1.24	3.0	52	3.68	4.5
36-60 hours	85	0.58	1.1	74	1.36	2.3
60-84 hours	142	0.78	2.6	9	1.31	0.3
84-120 "	59	1.60	2.7	27	1.48	0.9
Total gold excreted.			10.4%			8.2%

Table 15.

The concentrations of gold in certain additional tissues of rabbits 24 hours after the intravenous administration of 0.01mM./kg. gold chloride, without and with treatment with dimercaprol.

Treatment	Nil	50mg./kg. dimercaprol at 19 hours, and 12.5mg./kg. at 23hrs.
Rabbit	202	212
	µg. Au per g. fresh weight of tissue.	
Ribs	4.23	1.81
Vertebrae	2.98	0.97
Semilunar cartilage.	2.79	-
Joint Capsule	1.45	-
Lymph glands	4.95	2.31
Salivary glands	1.63	0.75
Thyroid	3.07	1.10
Pancreas	1.41	1.06
Adrenals	2.51	2.56
Thymus	1.29	0.63
Ovaries	4.24	-
Testes	-	0.80

PART III: Lead. Acute distributions.

Results are presented for the distribution and/or excretion of lead in twelve rabbits, all of which received a single intravenous dose of 0.01mM./kg. of lead acetate. Two of these rabbits received no further treatment, and six were treated with dimercaprol glucoside, as set out in Tables 16 and 17. One animal was treated with dimercaprol, two with 1:3-dimercaptopropanol, and one with 1:4-dithioerythritol, and the results are shown in Tables 19 and 20.

A further series of seven rabbits received a single intravenous dose of 0.1mM./kg. of lead acetate, and three of these were treated with dimercaprol and three with dimercaprol glucoside. Observations were made on the lead contents of blood, kidneys, and urine only, and the results obtained are given in Table 18.

All these animals were killed twenty-four hours after the administration of lead acetate.

The concentrations of lead in microgrammes per gramme of tissue and the percentages of the dose found/

found in various tissues and organs are shown in Tables 16 and 17, for those animals which received 0.01mM./kg. (2.07mg. Pb/kg.) of lead acetate, without and with treatment with dimercaprol glucoside. This dose of lead is well below the toxic level, and the animals appeared quite healthy up to the time of death.

Despite the variation occurring between animals treated in the same way, the following trends can be seen.

The control animals which received 0.01mM./kg. of lead acetate provided results which are generally consistent with those obtained by Ginsburg and Weatherall (1948), using a similar method. The main differences are that higher concentrations of lead were found in the kidneys in these animals, and a larger amount of lead was excreted in the urine, 1-4% as against 0.2-1%. It is probable that these differences can be attributed, at least in part, to the rate of injection of lead. Weatherall (personal communication) states that rapid injections of lead acetate were given in the experiments of Ginsburg and Weatherall.

High/

High concentrations of lead, 18-22µg. per gramme, occurred in the liver, which contained about 30% of the dose, and in the spleen, bone marrow, and epiphyses. Concentrations of 2-6µg. of lead per gramme were found in diaphyses, lungs, bile, and erythrocytes. The colon and small intestine contained less than 1µg. per gramme, but the concentration of lead was slightly higher in the gut contents. Skin, skeletal muscle, and plasma held only very small amounts of lead.

The experiments of Ginsburg and Weatherall dealt comprehensively with the effect of dimercaprol on the distribution of lead in rabbits after an intravenous dose of 0.01mM./kg. of lead acetate. For this reason, only one rabbit in the present studies was treated with dimercaprol (vide infra).

The administration of dimercaprol glucoside caused several changes in the distribution of lead. Decreases in the amounts of lead in the liver and erythrocytes were found. The quantities of lead in bone and kidneys also tended to decrease, but these changes were not very significant. The changes were accompanied by an increased urinary excretion/

Table 16/

Table 16.

The concentrations of lead in the tissues of rabbits 24-hours after the administration of 0.01mM./kg. of lead acetate, without and with treatment with dimercaprol glucoside.

Treatment with dimercaprol glucoside.	None		175mg./kg. at 1 hour and 55mg./kg. at 5 hours.			175mg./kg. at 19 hour and 55mg./kg. at 23 hours.		
Rabbit No.	381 ♀	374 ♀	373 ♀	375 ♀	382 ♂	376 ♂	379 ♂	383 ♂
Weight, kg.	1.98	1.59	1.63	1.68	1.31	1.53	1.31	1.66
	Microgrammes of lead per gramme fresh weight of tissue.							
Plasma	<0.02	<0.03	<0.02	0.75	<0.04	<0.01	<0.02	<0.02
Erythrocytes	3.96	1.86	1.35	-	0.61	0.92	1.30	0.40
Lungs	4.61	1.91	2.36	5.38	21.04	5.73	3.57	20.00
Small intestine	0.45	0.53	0.39	0.65	0.47	1.08	0.83	1.65
" " contents	1.87	1.14	2.72	1.20	1.35	1.35	1.73	1.72
Colon	0.83	0.78	0.23	1.13	0.49	0.93	-	0.97
Colon contents	3.95	2.94	5.09	13.12	8.13	3.95	1.97	17.53
Kidneys	23.46	10.99	1.87	6.66	24.58	2.84	8.79	16.95
Liver	18.85	21.64	12.75	14.68	12.59	20.20	10.60	16.54
Bile	5.84	3.02	28.36	33.32	-	25.09	10.01	37.22
Spleen	18.70	17.88	19.33	13.50	2.01	33.71	8.07	2.69
Skin	0.21	<0.03	<0.04	0.16	<0.03	0.07	<0.02	0.75
Bone Marrow	5.72	23.31	6.68	11.93	16.04	16.22	6.37	7.29
Epiphyses	11.64	13.57	6.23	4.64	11.37	9.00	12.62	11.37
Diaphyses	6.39	5.02	2.68	2.08	2.60	4.56	5.03	4.17
Skeletal muscle	<0.01	<0.01	<0.01	0.08	<0.01	0.06	-	<0.03

Table 17.

The percentages of the dose of lead in the tissues of rabbits 24-hours after the administration of 0.01mM./kg. of lead acetate, without and with treatment with dimercaprol glucoside.

Treatment with dimercaprol glucoside.	None		175mg./kg. at 1-hr and 55mg./kg. at 5 hours.			175mg./kg. at 19-hrs and 55mg./kg. at 23-hours.		
Rabbit No.	381 ♀	374 ♀	373 ♀	375 ♀	382 ♂	376 ♂	379 ♂	383 ♂
Weight, kg.	1.98	1.59	1.63	1.68	1.31	1.53	1.31	1.66
Plasma	0.04	<0.05	<0.04	1.5	<0.08	<0.02	<0.05	<0.04
Erythrocytes	4.6	2.7	1.4	-	0.8	1.3	1.8	0.4
Lungs	1.0	0.4	0.6	0.9	4.8	0.8	0.9	3.0
Small intestine	0.6	0.6	0.4	0.5	0.9	1.2	1.0	1.2
" " contents	1.0	0.4	0.7	0.3	0.3	0.2	0.2	0.4
Colon	0.2	0.2	0.1	0.2	0.1	0.3	-	0.1
Colon contents	2.0	0.9	1.5	0.9	1.9	0.5	0.8	2.5
Kidneys	8.0	3.2	0.6	2.9	8.9	1.0	3.2	5.7
Liver	33.8	26.3	14.3	16.9	22.5	24.7	18.6	21.2
Spleen	0.7	0.4	0.3	0.1	0.1	1.2	0.4	0.1
Skin	1.5	<0.2	<0.2	1.0	<0.2	0.4	<0.2	4.3
Bone marrow	8.3	26.2	6.5	11.5	16.4	15.6	6.2	7.0
Bone	16.5	14.5	7.8	6.0	7.6	13.2	14.6	12.1
Skeletal muscle	<0.3	<0.3	<0.3	2.0	<0.3	1.6	-	<0.7
Excreted, urine	4.1	1.4	17.8	31.5	28.1	10.4	25.4	14.8
Excreted, faeces	0.2	0.8	0.9	-	0.6	0.5	-	-
Injection site	7.6	0.1	26.9	0.3	0.4	1.4	2.1	0.5
Total recovery	90.1%	78.1%	79.8%	76.5%	93.4%	74.3%	75.2%	73.3%

excretion of lead, accounting for most of the metal lost from the tissues. A large increase in the biliary excretion of lead also occurred, about 0.6 per cent of the dose being found in the bile from the gall bladder, as compared with 0.1 per cent in the case of the control animals. The concentrations of lead in other tissues were not altered to any significant degree by dimercaprol glucoside treatment, nor was the faecal excretion affected, though a longer period of observation might have disclosed an increase, considering the enhanced biliary excretion of lead. The high concentrations of lead found in the lungs of rabbits Nos.382 and 383 were possibly due to the formation of some particular matter in the injection solution, with subsequent deposition of these particles in the lungs.

The main changes occurring after dimercaprol glucoside treatment are summarised in Fig.6. The concentrations of lead shown for the different organs and tissues, are the mean figures for both control animals and for all the animals receiving treatment. This tends to over-simplify the results/

FIG. 6a.

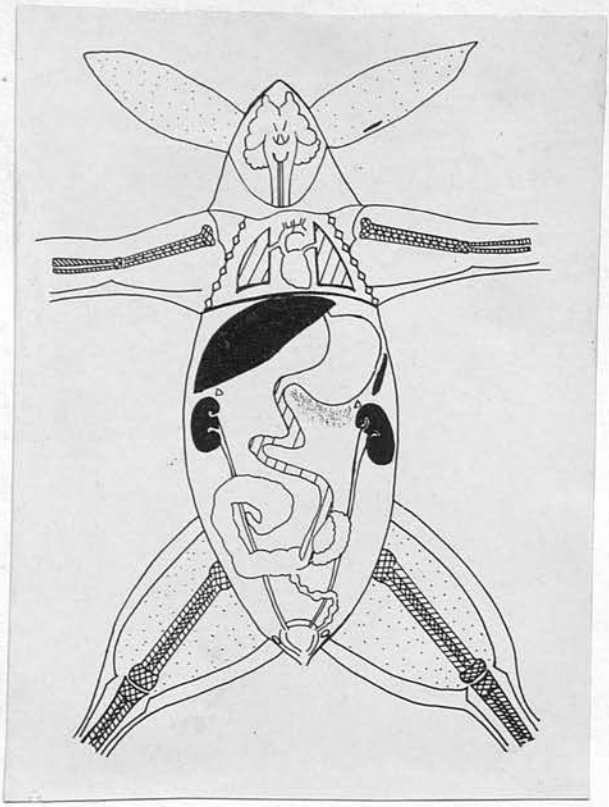


FIG. 6b.

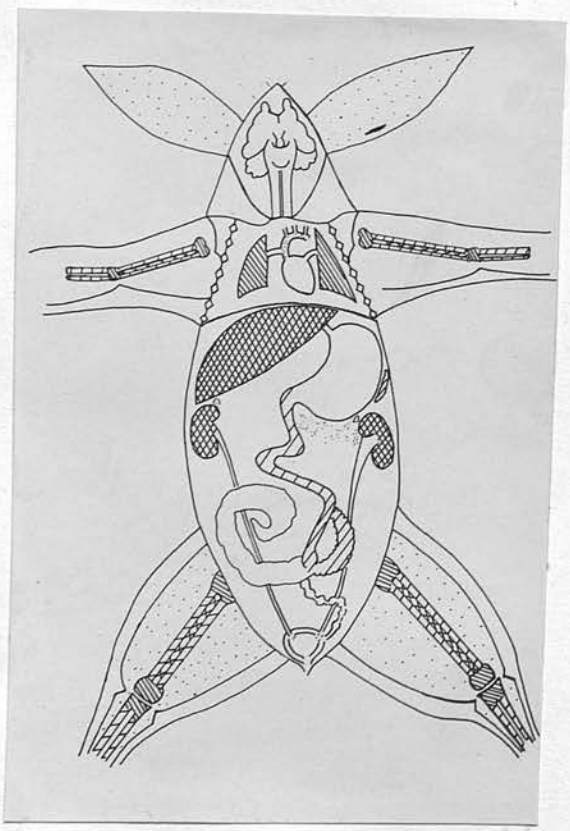
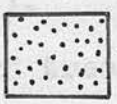


Fig. 6 (a). The mean concentrations of lead in various tissues and organs twenty-four hours after the intravenous administrat-ion of 0.01 mM./kg. of lead acetate.

Fig. 6 (b) The mean concentrations of lead in various tissues and organs twenty-four hours after the intravenous administrat-ion of 0.01 mM./kg. of lead acetate, and subsequent treatment with dimercaprol glucoside.



results, since early treatment with the glucoside caused a greater decrease in the lead content of the bones, and a greater increase in the urinary excretion of lead, than did late treatment.

The effect of treatment with dimercaprol was investigated in one experiment (rabbit No.377), sampling only those tissues containing high concentrations of lead in the control animals. The results are shown in Tables 19 and 20, and are consistent with those obtained by Ginsburg and Weatherall (1948). The main differences between the effects of treatment with the two dithiols, are the greater quantities of lead found in urine and bile after administration of the glucoside. It is difficult to determine from which tissues this increased loss of lead occurs, owing to the variation shown by the animals.

An intravenous injection of 0.1mM./kg. (20.7mg. Pb/kg.) of lead acetate is close to the median lethal dose for rabbits. The animals poisoned with this quantity of lead showed signs of considerable renal damage. All samples of urine contained/

contained quantities of casts, and haematuria occurred in some cases. The kidneys of the latter animals showed haemorrhagic areas. There was no relationship between the incidence of haematuria and the treatment given. Varying degrees of haemolysis were found by inspection of the plasma.

The distribution of lead after this dose of lead acetate is similar to that found after a dose of 0.01mM./kg., except that a higher proportion of the dose tends to occur in the bone marrow, and a smaller proportion in the erythrocytes (Weatherall, personal communication). The quantities of lead found in the blood of the one untreated animal (rabbit 427) are similar to the figures given by Weatherall, but the amounts in the kidneys and urine are somewhat higher (Table 18).

The results obtained from rabbit 423, which was treated with dimercaprol at one and five hours after the administration of lead acetate, are quite at variance with those of the other animals. No explanation/

Table 18/

Table 18.

The percentages of the dose of lead in blood, kidneys, and urine 24 hours after the intravenous administration of 0.1mM./kg. lead acetate, without and with treatment with dimercaprol or dimercaprol glucoside.

Treatment	Nil	Dimercaprol			Dimercaprol glucoside		
		50mg./kg. at 1 hr. and 12.5mg./kg. at 5 hours.		50mg./kg. at 19-hrs and 12.5mg./kg. at 23 hours.	175mg./kg. at 1-hr. and 55mg./kg. at 5-hours.		175mg/kg. at 19-hrs. and 55 mg./kg. 23-hours
Rabbit No.	427 ♀	420 ♀	423 ♂	426 ♀	421 ♂	422 ♂	424 ♀
Weight, kg.	1.69	1.72	1.64	1.66	1.90	1.67	1.60
Injection site.	0.1	0.8	2.7	<0.1	0.2	1.2	0.8
Plasma	<0.3	-	3.3	<0.3	-	<0.06	<0.01
Erythrocytes	0.8	-	3.1	<0.3	-	<0.06	0.4
Kidneys	1.1	-	2.1	0.4	-	0.8	0.5
Excreted, urine.	1.4	5.1	0.2	4.8	6.5	4.7	3.9

explanation can be offered for this finding. If this set of results is ignored, then a close similarity is found between the effects of dimercaprol and dimercaprol glucoside treatment. The excretion of lead in the urine was increased to 3-4 times that of the control animal. The concentrations of lead in the erythrocytes and the kidneys was decreased.

Although a smaller percentage of the dose was excreted than that obtained after administration of the smaller dose of lead acetate, with similar treatment, the actual weight of lead in the urine was higher - about 2mg. as compared with 0.2-0.5mg.

The results indicate that the effectiveness of dimercaprol glucoside in promoting the urinary excretion of lead was diminished to a larger extent by the increased dose of lead acetate than was that of dimercaprol. The significance of this finding is discussed later.

In two additional experiments (rabbits Nos.428 and 429), 1:3-dimercaptopropanol or 1:4-dithioerythritol were administered at one hour and five hours after the injection of 0.01mM/kg. of lead acetate. Again the amounts of lead in only the blood,/

blood, kidneys, and urine were studied, since these experiments were carried out mainly to test the ability of the dithiols to promote the urinary excretion of the metal. The results are presented in Tables 19 and 20. Both substances increased the excretion of lead, but 1:4-dithioerythritol tended to be less effective than dimercaprol. The rabbit treated with 1:3-dimercaptopropanol, however, excreted a larger percentage of the dose of lead than did any other animal in the present series of experiments. Nearly 42% of the dose was found in the urine of this animal, in spite of the very small dose which, because of the high toxicity of 1:3-dimercaptopropanol, was given. It should be noted that the excretion figures for the control animals (Table 17) were 1% and 4% of the dose.

This experiment was repeated (rabbit No.430) and this time about 27% of the dose occurred in the urine. Even this result compares favourably with those obtained after treatment with dimercaprol glucoside, and in the latter case 7-8 times the number of molecules of dithiol was administered.

Little/

Table 19/

Table 19.

The concentrations of lead in various tissues 24-hours after the intravenous administration of 0.01mM./kg. of lead acetate, with subsequent treatment with dimercaprol, 1:3-dimercaptopropanol or 1:4-dithioerythritol.

Treatment	Dimercaprol 50mg./kg. at 1-hr and 12.5mg./kg. at 5-hrs.	1:3-dimercaptopropanol 12.5mg./kg. at 1-hr and 3.2mg./kg. at 5-hrs.	1:4-dithioerythritol 75mg./kg. at 1-hr and 19mg./kg. at 5-hrs.
Rabbit No.	377 ♂	428 ♂	429 ♂
Weight, kg.	1.82	1.73	1.46
	µg. of lead per g. fresh weight of tissue.		
Plasma	<0.02	<0.15	<0.43
Erythrocytes	1.25	3.92	5.94
Liver	13.35	-	-
Kidneys	4.71	6.44	8.30
Bone marrow	20.41	-	-
Epiphyses	8.30	-	-
Diaphyses	4.07	-	-

Table 20.

The percentages of the dose of lead in various tissues 24-hours after the intravenous administration of 0.01mM./kg. of lead acetate, with subsequent treatment with dimercaprol, 1:3-dimercaptopropanol or 1:4-dithioerythritol.

Treatment	Dimercaprol 50mg./kg. at 1-hr and 12.5mg./kg. at 5-hrs.	1:3-dimercaptopropanol 12.5mg./kg. at 1-hr and 3.2mg./kg. at 5-hrs.		1:4-dithioerythritol 75mg./kg. at 1-hr and 19mg./kg. at 5-hrs.
Rabbit No.	377 ♂	428 ♂	430 ♀	429 ♂
Weight, kg.	1.82	1.73	1.54	1.46
Plasma	<0.04	<0.2	-	<0.9
Erythrocytes	1.4	5.9	-	4.8
Liver	20.7	-	-	-
Kidneys	1.3	2.1	-	3.2
Bone Marrow	22.1	-	-	-
Bone	8.2	-	-	-
Excreted, urine	17.4	41.7	26.9	10.1
Injection site	3.2	0.2	0.4	0.7

Little information is available to show from which tissues this loss of lead occurred, but apparently the lead in the erythrocytes was not decreased as it was in the animals treated with dimercaprol or dimercaprol glucoside. The lead content of the kidneys was smaller than that of the untreated animals, and the lead in the plasma remained of negligible quantity.

The urine of rabbit No.428 contained casts presumably due to the high concentration of lead which must have passed through the renal tubules, but the kidneys themselves were of normal appearance. Apart from this, rabbit No.428 showed no signs of toxic effects, but rabbit No.429 developed tremors, becoming almost mild convulsions at times, after the first injection of 1:4-dithioerythriol. One rabbit (not otherwise reported) developed convulsions and died within thirty minutes after an injection of 150mg./kg. of 1:4-dithioerythriol, (twice the dose administered to rabbit No.429).

LEAD: Sub-acute distributions.

The following experiments were carried out in collaboration with Dr. M. Ginsburg and Dr. M. Weatherall, and have already been published (Adam, et al. 1949).

Results are presented for the distribution and excretion of lead in seven rabbits, all of which received, by intravenous injection, a single dose of lead acetate containing 2.07mg. of lead per kg. of body weight. Some of the rabbits were treated with dimercaprol or with parathyroid extract or with both, as indicated in Table 21, and all were killed after twenty-one days. The parathyroid extract, which is believed to mobilize lead from bones (Hunter and Aub, 1927), was given in the morning. The dimercaprol was given at the same time, though injected at a different site, and again four hours later, in order to cover the period in which the greatest mobilization of lead might be expected.

The concentration of lead in microgrammes per gramme of wet weight of tissue and the percentage of the dose found at death (i.e., three days after the end of treatment) in various organs and calculated

for/

for various tissues are shown in Tables 21 and 22. Only one portion of the intestine, the colon, was sampled. The rest of the alimentary canal was the largest amount of tissue not examined in these animals, but probably did not contain more than 1 or 2 per cent of the dose.

Of the lead which remained in the body, regardless of treatment and apart from that which remained at the site of injection (notably rabbit 399), 60 to 95 per cent was found in the bones. No other single tissue accounted for more than a few per cent of the dose, and only the liver and bone marrow contained more than 2 per cent consistently. Similarly, the highest concentrations were found in the bones, bone marrow, and liver, which generally contained 1-10µg. of lead per g. of tissue. Other tissues rarely contained more than 1µg. per g. The concentrations in blood cells, lungs, kidneys, bile from the gall bladder, and skin tended to be above 0.1µg. per g. whereas those in skeletal muscle, the alimentary canal, and brain tended to be below this level.

Inspection of the results shows no striking differences between differently treated animals. The concentrations/

Table 21.

The effect of treatment with dimercaprol and parathyroid extract on the concentration of lead in the tissues of rabbits 21 days after the intravenous administration of lead acetate (2.07mg. Pb/Kg.)

	Microgrammes of lead per gramme fresh weight of tissue.						
Treatment with parathyroid extract	None	None		8u/kg/day on days 15, 16, and 17.		8u/kg/day on days 15, 16, and 17.	
Treatment with dimercaprol	None	12.5mg./kg. twice daily, days 15-18		None		12.5mg./kg. twice daily, days 15-18	
Rabbit No.	398 ♂	378 ♀	396 ♂	384 ♂	397 ♀	380 ♀	399 ♂
Init. wt. kg.	2.02	1.90	1.82	1.70	2.06	1.90	2.02
Final wt. kg.	1.90	1.60	1.65	1.80	1.80	1.80	-
Plasma	<0.16	<0.09	<0.22	<0.10	<0.13	<0.05	<0.16
Blood cells	0.62	0.19	0.34	0.21	<0.27	0.07	0.35
Spleen	<0.64	0.30	<1.30	<0.90	<0.83	<0.23	<1.50
Bone marrow	3.50	<0.25	7.0	4.6	4.2	1.10	2.70
Liver	1.9	0.62	2.0	3.3	2.2	4.7	1.4
Bile	<0.64	1.0	<2.9	0.33	<1.0	0.59	<2.21
Pancreas	-	<0.08	-	<0.45	-	<0.15	-
Colon	<0.07	0.01	<0.10	0.03	<0.08	0.01	<0.12
Colon contents	<0.30	0.03	<0.12	0.04	<0.15	0.11	-
Kidneys	0.67	0.16	0.45	0.53	0.55	0.27	0.50
Adrenals	-	<1.14	-	<2.07	-	<0.87	-
Gonads	-	<0.64	-	<0.64	-	<0.55	-
Seminal vesicles	-	-	-	<0.11	-	-	-
Uterus	-	<0.04	-	-	-	<0.06	-
Lungs	<0.13	0.83	1.86	0.02	0.34	<0.02	0.20
Skeletal muscle	<0.09	<0.01	<0.09	<0.02	0.13	<0.01	<0.07
Diaphysis	5.1	3.8	2.6	7.0	3.5	4.4	1.4
Epiphysis	12.9	5.7	8.0	10.9	5.2	3.5	2.5
Ribs	19.0	6.5	9.7	10.0	7.8	6.4	1.5
Vertebrae	-	6.2	-	10.0	-	5.4	-
Skull vault	12.8	10.4	5.5	7.8	5.0	12.5	2.6
Brain	<0.13	0.12	<0.12	0.10	0.15	<0.02	0.11
Skin	0.20	0.11	<0.15	0.10	<0.14	0.10	0.30

Table 22.

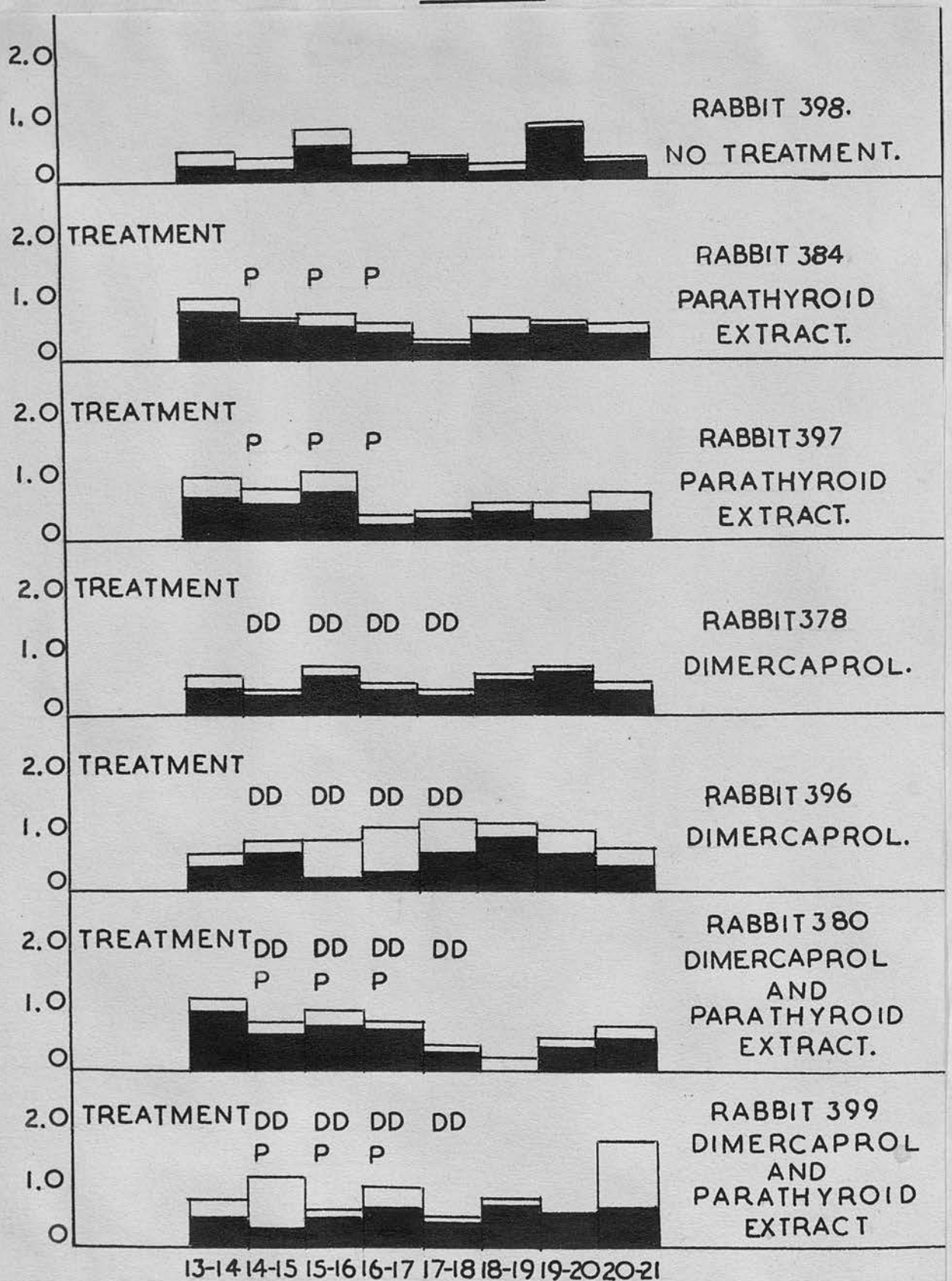
The effect of treatment with dimercaprol and parathyroid extract on the distribution of lead in rabbits twenty-one days after the intravenous administration of lead acetate (2.07mg. Pb/Kg.)

		Percentage of dose in entire organ or tissue					
Treatment with parathyroid extract	None	None		8u/kg/day on days 15, 16 and 17.		8u/kg/day on days 15,16 and 17.	
Treatment with dimercaprol	None	12.5mg./kg.twice daily,days 15-18		None		12.5mg./kg. twice daily,days 15-18	
Rabbit No.	398 ♂	378 ♀	396 ♂	384 ♂	397 ♀	380 ♀	399 ♂
Init. wt. kg.	2.02	1.90	1.82	1.70	2.06	1.90	2.02
Final wt. kg.	1.90	1.60	1.65	1.80	1.80	1.80	-
Plasma	<0.30	<0.24	<0.45	<0.22	<0.30	<0.11	<0.31
Blood cells	0.86	0.20	0.45	0.24	<0.30	0.08	0.48
Spleen	<0.02	0.01	0.02	<0.02	0.02	<0.01	<0.02
Bone marrow	3.3	<0.38	6.8	4.4	4.0	0.99	2.5
Liver	2.2	0.70	2.0	4.5	2.8	5.4	1.3
Pancreas	-	<0.01	-	<0.01	-	<0.01	-
Colon	<0.02	0.01	<0.02	0.01	<0.02	0.01	<0.02
Colon contents	<0.04	0.01	<0.05	0.01	<0.04	0.04	-
Kidneys	0.18	0.04	0.14	0.16	0.13	0.07	0.14
Adrenals	-	<0.01	-	<0.06	-	<0.01	-
Gonads	-	<0.01	-	<0.02	-	<0.01	-
Lungs	<0.02	0.02	0.26	0.01	0.05	<0.01	0.03
Skeletal muscle	<2.2	<0.68	<2.3	<0.58	3.3	<0.28	<1.8
Bone	35.8	19.2	18.7	27.4	15.7	18.7	5.7
Brain	<0.02	0.02	<0.02	0.02	0.03	<0.01	0.02
Skin	1.2	0.61	<0.87	0.60	<1.00	0.57	1.8
Injected ear	0.23	0.02	0.14	0.41	0.02	0.59	33.3
Total in carcass	44%	21%	29%	39%	26%	26%	45%
Excreted:							
Urine, days 0-14	10.8	11.3	16.9	11.8	12.3	14.9	9.2
Urine, days 14-21	1.1	0.8	3.2	0.8	1.7	1.3	3.1
Faeces, days 0-14	36.1	33.9	17.1	28.9	32.7	27.1	8.0
Faeces, days 14-21	5.7	2.7	3.6	2.7	2.0	3.1	4.9
Total excreted	54%	49%	41%	44%	49%	45%	25%
Total accounted	98%	70%	70%	83%	75%	71%	70%

concentrations in bone were higher in the untreated animal than in any others. Interpretation of this difference is confused by the variability of the concentration in different bones, by the large deposit of lead at the site of injection in rabbit 399, and by the variation in the amount of lead excreted before treatment was started. Since the treated animals did not show any definite increase in the amount of lead outside the skeleton, the evidence that appreciable quantities of lead was removed from the bone by any of the treatments is unconvincing. Other tissues showed no consistent differences which could be related to the treatments.

The amounts of lead excreted before and after treatment are shown in Table 22. Faecal excretion was generally two to three times greater than the urinary excretion, and the amounts of lead eliminated appeared to depend to some extent on the quantity of excreta passed. Treatment with dimercaprol on the fifteenth and subsequent days after the administration of lead, was followed by an increase in the concentration of lead in the urine and faeces. This was accompanied/

FIG. VII.


2.0
1.0
0
RABBIT 398.
NO TREATMENT.
TREATMENT
P P P
RABBIT 384
PARATHYROID
EXTRACT.
TREATMENT
P P P
RABBIT 397
PARATHYROID
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TREATMENT
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TREATMENT
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RABBIT 396
DIMERCAPROL.
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RABBIT 380
DIMERCAPROL
AND
PARATHYROID
EXTRACT.
TREATMENT
DD DD DD DD
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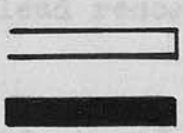
RABBIT 399
DIMERCAPROL
AND
PARATHYROID
EXTRACT
13-14 14-15 15-16 16-17 17-18 18-19 19-20 20-21
TIME IN DAYS AFTER INJECTION

Fig. 7.

The effect of dimercaprol and parathyroid extract on the excretion of lead in the urine and faeces 15-18 days after the intravenous administration of lead acetate (2.07 mg. Pb/kg).

Ordinates: Amount of lead excreted as a percentage of the dose administered (1 per cent= 20.7 µg. Pb/kg. body weight). Abscissae: Number of days after the injection of lead acetate . D= 12.5 mg./kg. dimercaprol injected intramuscularly. P = 8 units /kg. parathyroid extract injected intramuscularly.



URINARY EXCRETION.

FAECAL EXCRETION.

accompanied by a reduction in the quantity of excreta, so that there was no increase in the total amount of lead excreted. A slight fall of doubtful significance occurred in the total quantity of lead excreted by the rabbits treated with parathyroid extract or with dimercaprol and parathyroid extract, probably owing to the decrease in the quantity of excreta which also occurred in these rabbits. Rabbit 399 developed acute retention of urine during the days preceding death, when over 400ml. of urine was found in the bladder. No cause for this retention was apparent, but it accounts for the large amount of lead recorded as excreted on the twenty-first day.

The effects of the treatments on the elimination of lead in the urine and faeces are shown in Fig.7.

DISCUSSION

METHOD:

The method used in these experiments is simple in principle, and the estimations, as compared with chemical analyses, are rapid and sensitive. These advantages afforded by the use of radio-active isotopes are well known. Nevertheless, there are certain disadvantages which must be considered; and the extensive sampling of tissues, and the slow rate of ashing, rendered the work laborious and time-consuming.

The effect of the radiations emitted by the isotopes on the animals used in these experiments is unknown, but the possibility exists that some effect did occur. However, the general similarity between the results obtained here and those of previous workers who had used chemical methods indicates that any such effect must have been small.

A further disadvantage is that isotopes with short half-lives limit the lengths of the distribution periods which can be investigated. Fortunately,/

Fortunately, in the case of lead, the slowly-decaying isotope radium D was available for use in the distribution periods of more than one day; but no isotope of gold, other than Au^{198}, is available and the maximum distribution period which can be attempted is about a week. The period could be extended by increasing the activity of the injection solution, but this would afford a greater risk of radiation effects in the animals.

A new technique which has been reported recently (Tobias and Dunn, 1949) obviates these difficulties. This method involves the neutron irradiation of ashed samples taken from poisoned animals in the usual manner. In this way, the metal is rendered radioactive, and available for estimation, after the animal has been killed. The distribution period can be as long as desired, since only the stable element is administered to animals. However, the method requires permanent access to a pile, and facilities for the radio-chemical separation of the element.

Data concerning the distribution and excretion of a poison under the influence of an antidote may provide considerable information regarding the way in/

in which the antidote acts. Excretion of the poison may be promoted or its transfer to less susceptible tissues facilitated.

Such results are conditioned to a large extent by variables such as dosages and routes of administration of poison and antidote, the periods over which observations are made, and the delay between poisoning and initiation of treatment. Furthermore, large variations occur between animals treated in the same way. A satisfactory investigation, demonstrating the effects of these variables, would involve the use of large numbers of animals and considerable time and labour. From this point of view, the present work is incomplete.

In describing the changes occurring after the administration of the antidote, it should be remembered that part of these effects may be due to actions of the antidote on the animals. No doubt the changes mainly depend on the relative dissociability of the compounds formed between the metal and tissue constituents and between the metal and the dithiol, and on the subsequent behaviour of the metal-dithiol/

dithiol compound. The former factor will be influenced by the concentration of dithiol in any given tissue, but no data are available on the distributions of the dithiols in the present experiments.

With due recognition of these points, the results of the present series of experiments have afforded certain conclusions which are discussed in the following sections.

Mercury/

Mercury.

The literature describing the distribution and excretion of mercury in animals is small in quantity, and meagre in information. Very small amounts of the soluble compounds of mercury kill animals within a short period of time, and hence a sensitive analytical method is necessary. A satisfactory and fairly comprehensive study of the distribution of mercury in human cadavers after death from mercuric chloride poisoning, was made by Sollmann and Schreiber (1936). In these cases, ofcourse, comparatively large quantities of mercury were available for estimation. They reported that mercury could be found in the following sites, in order of decreasing concentration: kidneys, liver, spleen, intestine, lungs, heart, skeletal muscle, brain, blood. Very little mercury was excreted, for in such fatal cases anuria develops early in poisoning.

Lomholt (1924) investigated the distribution and excretion of mercury in rabbits, after giving a series of intramuscular injections of mercuric benzoate, mercuric salicylate, calomel, or metallic mercury. Only one rabbit was studied per compound, and the animals were killed a variable number of days after the/

the administration of mercury. Large quantities of the mercury were unabsorbed, but the most soluble compound, mercuric salicylate, gave the following results after nine days: 29% of the dose excreted, and 34% unabsorbed; the kidneys held much the highest concentration of mercury, followed by liver, intestines and contents, lungs, and blood, in that order.

Young et al. (1930) also studied the distribution of mercury in rabbits after the administration of mercuric salicylate, but gave very scanty data. They reported that 'during active excretion' the quantities of mercury in the tissues occurred in this order of decreasing concentration: bone, skeletal muscle, kidneys, liver; and 'after active excretion': bone, liver, skeletal muscle, kidneys. In the latter case, bone contained by far the highest concentration of mercury. No data regarding dosage and route of administration, or a definition of the term 'active excretion' were given.

Literature containing older analyses was unfortunately inaccessible, but Sollmann and Schreiber (1936) quote Lomholt (1928) as describing, in a review of/

of the fate of mercury in the body, a similar general distribution to that which they had found.

The radio-active tracer method is well suited to application in such a problem as this, where only minute quantities of a substance are available for estimation. Although the mercury used was of comparatively low specific activity, quantities of the order of 0.1µg. in an average sample could be detected. This enabled extensive and accurate investigations of the fate of a single intravenous dose of mercuric chloride to be made.

The results are largely in accord with those obtained by Lomholt, and by Sollmann and Schreiber, described above. Quantitative differences occur because of higher doses and slower rates of absorption in the studies of these authors. The only qualitative differences are that Sollmann and Schreiber found larger amounts of mercury in the heart and spleen, but this may be related to the route of administration.

The observations of the present studies are not consistent with the data of Young et al. (1930). These authors suggested that the distribution of mercury/

mercury was very similar to that of lead, but the results shown in Tables 2 and 16 indicate that these elements differ markedly in their affinities for particular tissues. For instance, lead was found to concentrate in the bone, bone marrow, and spleen, whereas only small quantities of mercury occurred in these sites.

The slow excretion of mercury, predominantly in the urine, has been noted in all these works, as well as in the present studies where less than two-thirds of the dose of mercury was excreted in two weeks.

The striking decreases which occurred in the concentrations of mercury in the kidneys after treatment with dimercaprol, account for the diminished renal injuries noted by Ginzler (1946) and by Gilman et al. (1946a). These authors found that delay in treatment greatly decreased the protection afforded to the kidneys by dimercaprol, yet the present experiments show that the quantities of mercury in the kidneys are still diminished by treatment begun several days after poisoning. Presumably after a time depending on the dose of mercury, irreversible changes take place as the result of the prolonged contact/

contact of the mercury with susceptible enzymes, and the lesion remains although the mercury is removed.

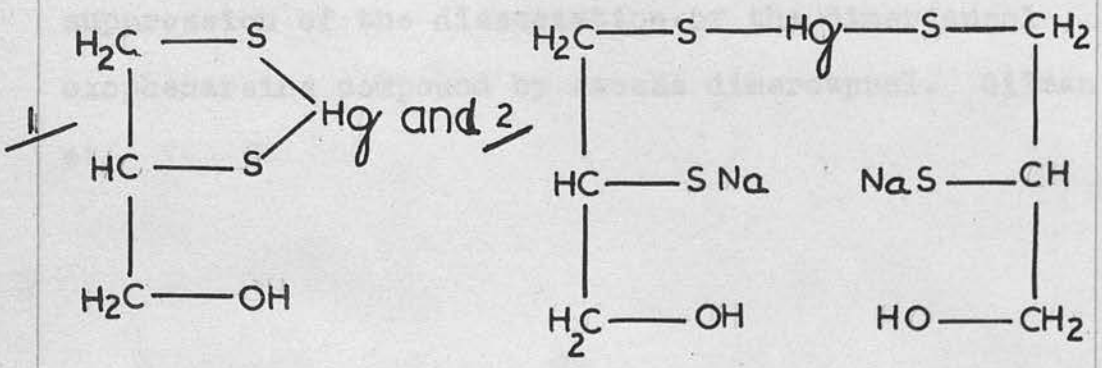
This decrease in the concentration of mercury in the kidneys has also been reported very recently by Fitzsimmons and Kozelka (1950) who administered 10mg. of mercuric chloride to Rhesus monkeys by the oral route, and 0.5mg./kg. of mercuric chloride intraperitoneally in rats, and subsequently treated the animals with dimercaprol. These authors state that the mercury contents of other tissues were raised after dimercaprol, but do not give any details. They also found that the rats excreted more mercury in the urine after treatment with dimercaprol, but that the faecal excretion was unchanged.

This increased urinary excretion of mercury was found in the present work, and it occurred even when treatment was delayed until nine days after the administration of mercuric chloride. This effect suggests that dimercaprol therapy might prove beneficial in more chronic types of mercury poisoning. Evidence was obtained that the increase in the excretion of mercury was transient in spite of continued treatment, but possibly intermittent courses of dimercaprol therapy/

therapy might induce repetitions of the initial effect.

The fact that continued treatment does not maintain a supra-normal excretion of mercury although a considerable amount of the metal remains in the body, indicates that the mercury fixed by the tissues can be divided into two categories. One section of the mercury is comparatively labile, and readily leaves the tissues to combine with dimercaprol; the other section must form a less readily dissociated compound with some tissue constituent. This raises the possibility of an alternative chemical configuration which would form a more stable compound with mercury than does dimercaprol.

Gilman et al. (1946a) have shown that dimercaprol can react with mercuric chloride in vitro to form at least two different complexes. From a consideration of the molecular proportions in which the substances combined, the following formulae were suggested:

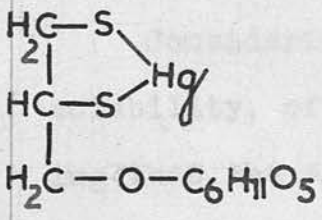


The latter compound was soluble, and was considered to be the more likely of the two to occur in vivo. It was also stated that the second compound was more stable than the first. This is surprising, since dithiols form more stable cyclic compounds with arsenic than do monothiols, which would form just such an open-chain structure as is suggested for the second complex. However, it is difficult to present any suitable alternative formulae.

These workers also found that the toxicity of the second complex, when administered to rabbits, was equal to that of mercuric chloride. Obviously, the excess of dimercaprol usually administered in mercury poisoning must diminish this toxicity in some way. The effect is reminiscent of that of the dimercaprol oxophenarsine compound, which has a toxicity equal to that of oxophenarsine itself, but if additional dimercaprol is administered the toxicity is reduced to one-fifth (Peters and Stocken, 1947). The latter authors ascribe this effect to suppression of the dissociation of the dimercaprol-oxophenarsine compound by excess dimercaprol. Gilman et/

et al. on the other hand, believe that the corresponding effect with mercury is due to the additional dimercaprol reacting with mercury released from the complex by intracellular oxidation of dimercaprol. However, the evidence for such destruction of dimercaprol is controversial (Webb and van Heyningen, 1947; Barron et al. 1947).

Support was given to the hypothesis of Gilman et al. by the toxicities of the compounds formed by dimercaprol glucoside and mercury. These compounds result from similar in vitro reactions to those between dimercaprol and mercury, and similar formulae were suggested:



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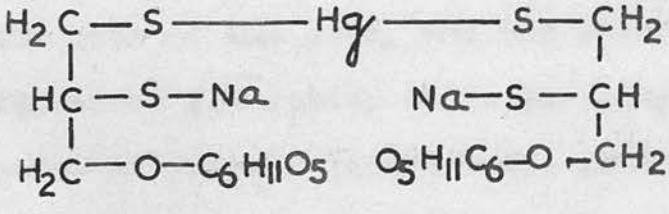

2

The toxicity of the first complex is about half, and that of the second about one-eighth that of mercuric chloride. Yet the mercury dissociates more readily from this first complex than from the second dimercaprol/

dimercaprol compound. Gilman et al, suggested that this was due to the extracellular distribution of the glucoside, with consequent immunity to oxidation.

The results reported here support to some extent the hypothesis of such a distribution of dimercaprol glucoside. The mercury level in the plasma was consistently raised after treatment with the glucoside, but not after dimercaprol therapy. It is difficult, however, to describe the increased biliary excretion of mercury as an extracellular effect.

Further evidence on this hypothesis is not given by the present studies, since the distributions of the preformed complexes were not studied.

Considering the size of the dose, and the water-solubility, of dimercaprol glucoside, it is surprising that the excretion of mercury in the urine after treatment with this substance was less than that obtained after dimercaprol therapy. It was considered that the initial large dose of dimercaprol glucoside might possibly have directed mercury to the kidneys in such high concentrations that damage resulted; but in one experiment (rabbit 407) the treatment was altered so as to give three similar medium-/

medium-sized doses of the glucoside, but this did not alter the quantity of mercury excreted. It should also be noted that when dimercaprol glucoside was administered immediately after the mercury, the quantities of metal in the urine after one hour were slightly higher than those obtained after dimercaprol treatment at corresponding times.

Possibly some type of renal damage occurs within an hour after the administration of mercury, and can be alleviated by dimercaprol but not by dimercaprol glucoside treatment, but this conception has little factual support.

Nevertheless, Gilman et al. (1946a) found dimercaprol glucoside to be as effective an antidote in mercury poisoning as was dimercaprol, in equimolecular doses. As even larger doses of mercury were administered in those experiments, it must be assumed that the mercury retained in the plasma by dimercaprol glucoside is as effectively non-toxic as if it had been excreted in the urine. Possibly this plasma mercury would have been found to diminish, and the metal excreted in the urine increased, had the observations been prolonged beyond 24 hours. In any case, the/

the fact that the plasma mercury was maintained at that level suggests that either the complex was attached to plasma proteins, or else that the renal tubules tended to reabsorb it. Experiments studying the renal clearance of mercury under these conditions might throw some light on the problem.

Gold/

Gold.

In the case of gold, there is rather more information available regarding the tissue distribution of this element, possibly because comparatively large amounts of gold can be administered to animals without causing serious toxic effects and the chemical methods of analysis appear to be quite satisfactory.

Orestano (1932) injected 19mg. of gold/kg. as gold chloride, intravenously in rabbits, and after five days found 3mg. of gold in the kidneys, 0.5mg. in the liver, 0.4mg. in bone marrow, 0.2mg. in the spleen, and only a trace in the lungs. Leulier and Béruard (1938) reported distributions of gold in rabbits over various periods, from a few minutes to one month after the intravenous injection of 40mg. of gold/kg. The liver, spleen, and lungs contained large amounts of gold at first, but the concentration in the lungs decreased very rapidly. Only small quantities of gold occurred in the kidneys, and very little in the plasma. The smaller gold content of the kidneys, as compared with Orestano's findings, may/

may be related to the different compound of gold used (calcium aurothioethanesulphate).

Block et al. (1941, 1942) administered 1mg. of gold, in the form of various therapeutic preparations, per day for fourteen days to rats by intramuscular injection. One day after the last injection, high concentrations of gold were found in the kidneys, liver, and spleen, and only small amounts in the lungs and heart. Considerable quantities of gold remained in the plasma, but none could be detected in the blood cells.

The radio-active isotope, Au^{198}, has been utilised previously to trace the tissue distribution of gold. Ely (1940) administered gold chloride, containing Au^{198}, to rats by the oral, intraperitoneal and subcutaneous routes. Very little absorption occurred from the intestine during the first 24 hours, but within three days most of the dose had been absorbed, and a large proportion was found in the kidneys. The adrenals, lungs, lymph nodes, liver and spleen also contained rather large amounts of gold, but very few details were given and only four animals/

animals were investigated.

Thirty days after the intravenous injection of 10mg. of gold, as gold sodium thiosulphate, in a mouse, concentrations of 0.4mg. of gold per g. to 0.005µg. per g. were found in the following tissues in order of decreasing magnitude: liver, ovaries, thymus, adrenals, lungs, lymph nodes, spleen, kidneys, heart, bone, pancreas, skin, gall bladder, tendon, muscle, blood cells, brain, gut, plasma. A recovery of 23% of the dose was obtained, and the rest of the gold was assumed to have been excreted (Tobias and Dunn, 1949).

The excretion of gold is generally slow. Leulier and Béruard, in the experiments quoted above, found only 8% of the dose excreted in the urine in 25 days. After the administration of 50mg. of gold, as aurothioglucose, to a patient, about 14% of the dose was excreted in the urine in 7 days, and about 4% in the faeces (Kent and McCance, 1941). Clinical reports state that about 20% of the dose is excreted in a week after the administration of 100mg. of gold, but that excretion may continue for several months (Freyberg et al. 1943; Comroe, 1945).

Rats/

Rats appear to excrete gold more readily than do rabbits or humans, for Block et al. (vide supra) reported urinary excretions of 68% of the dose after administration of sodium succimidoaurate and 41% after gold sodium thiosulphate. Less soluble gold compounds, such as gold calcium thiomalate, tended to be excreted more in the faeces than in the urine.

The present experiments indicated that, in rabbits, about as much gold was excreted in the faeces as in the urine a few days after the injection of gold chloride. They also confirmed the slow rate of excretion of gold in this species. The results of the tissue distributions are in general agreement with those obtained by previous workers. The concentrations of gold in the plasma are notably high, as found by Block et al. (1941), but in contrast to the findings of Leulier and Béruard (1938) and Tobias and Dunn (1949).

The low recoveries, and their possible cause, obtained in the present experiments have been discussed already. For two reasons, it is felt that the results are not so marred by the poor recoveries as to prohibit their inclusion in this thesis. They are/

are not incompatible with previous work on untreated animals. The error of the duplicate estimations is sufficiently low to suggest that similar fractions of gold were lost from each sample; therefore the results from treated and untreated animals are likely to be comparable, fulfilling the main purpose of the experiments. Nevertheless, it is desirable that the work should be repeated when the cause of the loss of gold has been determined.

The changes occurring after the administration of dimercaprol consisted mainly of a decrease in the gold content of the blood and an increase in the urinary excretion of the element. The excretion was almost doubled, but still consisted of only 6-9% of the dose after twenty-four hours. This effect has also been noted by Kuzell et al. (1948), after intravenous administration of gold sodium thiosulphate or gold chloride in rabbits, and subsequent treatment with dimercaprol.

Unfortunately, dimercaprol glucoside was not available for investigation at the time of these experiments. 1:3-dithiols have not been tested as antidotes/

antidotes to poisoning with gold, but it is possible that gold would combine more readily with these substances than with 1:2-dithiols, in a similar manner to lead (vide infra). Levey and Smyth (1947) analysed the complexes formed between dimercaprol and gold sodium thiosulphate or gold sodium thiomalate. In both cases, two atoms of gold were present for each molecule of dimercaprol, indicating an inability to form a cyclic compound like that postulated for the arsenic-dimercaprol complex.

Levey and Smyth also found that the administration of dimercaprol increased the survival-time of rats injected subcutaneously with 75mg. of gold/kg. as gold sodium thiosulphate, but some of the animals died of renal damage later. This is reminiscent of the effect of dimercaprol in cadmium poisoning (Gilman et al. 1946b; Tepperman, 1947). However, no direction of gold by dimercaprol towards the kidneys could be found in the present experiments, possibly because of the different species, dosages, and route of administration used.

Lead/

Lead: Acute distributions.

In contrast to the cases of gold and mercury, the fate of lead in the body has been the subject of extensive investigations. Since comparatively large amounts of lead can be administered to animals without causing early death, and adequately sensitive chemical methods have been available for some years, a number of satisfactory studies have been carried out. The radio-active isotopes of lead have been used previously (Lomholt, 1924; Behrens, 1925; Ginsburg and Weatherall, 1948; etc.) for tracing the metabolism of lead by similar methods to that used in the present studies, and by means of autoradiographs.

Because of the abundance of data which has accrued from these works, only those results immediately applicable to the present discussion will be considered; that is, those in which rabbits were poisoned by the intravenous administration of lead salts.

Forty-five minutes after the injection of 30 or 70.7mg. of lead/kg. as lead nitrate, about 25% of the dose/

dose was found in the blood, 35% in skeletal muscle, 5% in the liver, and 5% in the bones (Schütz and Bernhardt, 1925). At one hour and six hours after the administration of 2mg. of lead/kg. as the acetate, Ginsburg and Weatherall (1948) found 50% of the dose in the liver, and high concentrations of lead in the spleen, bone marrow, kidneys, lungs, and bone. Weyrauch (1931) obtained similar results sixteen hours after administering 12mg. of lead/kg. as the nitrate. By twenty-four hours, little further change occurred, and about 1% of the dose had been excreted in the urine (Ginsburg and Weatherall).

As has been stated already, the results obtained in the present series of experiments were consistent with those reported by Ginsburg and Weatherall in the case of the 0.01mM./kg. dose of lead, and by Weatherall for the 0.1mM/kg. dose.

Treatment with dimercaprol glucoside greatly increased the urinary and biliary excretion of lead. These effects also occurred after the administration of dimercaprol (Ginsburg and Weatherall, 1948, 1949), but in that case were of smaller magnitude. After raising/

raising the dose of lead acetate to 0.1mM./kg. and retaining the same treatment with dimercaprol or dimercaprol glucoside, the proportion of the dose excreted in the urine was diminished, though the actual weight of lead excreted was increased. It is interesting that, under these conditions, dimercaprol and dimercaprol glucoside were approximately equally effective in increasing the urinary excretion of lead. Possibly this decreased efficiency of the glucoside in comparison to dimercaprol after a large dose of lead can be explained by a greater proportion of the dose of lead occurring in intracellular sites, to which the glucoside is unable to penetrate. However, in contrast to the results obtained with mercury, the lead content of the plasma was not observed to increase after administration of dimercaprol glucoside; and no other evidence of an extracellular distribution was obtained.

In spite of the reports by some authors (Braun et al. 1946; Germuth and Eagle, 1948) of a potentiating action of dimercaprol in acute lead poisoning, no direction of lead by dimercaprol to some susceptible organ has been observed. The investigations on the/

the effect of dimercaprol on the mortality-rates of animals poisoned with lead have been mentioned briefly in the Introduction. It seems likely that in those cases in which dimercaprol has potentiated the action of lead, rapid mobilization of that element from subcutaneous or intraperitoneal depots has taken place. Germuth and Eagle (1948) found that a preformed complex of lead and dimercaprol was of equal toxicity to that of lead acetate itself. This is reminiscent of the toxicities of the oxophenarsine - and mercury-dimercaprol compounds mentioned previously (p.83). Possibly in the case of lead also, excess dimercaprol has a protective action. From a study of the dosages of lead and dimercaprol used in these tests of dimercaprol as an antidote, the impression is gained that where the two substances are likely to meet in such proportions that more than one molecule of dimercaprol is present for each atom of lead, then the action of dimercaprol tends to be beneficial. Certainly such an action has been found in those cases where indicators of poisoning, other than death, were studied, and hence smaller doses of lead were/

were administered (Weatherall, 1948; Chiodi and Sammartino, 1948; Mack, 1948).

While arsenic probably forms a very stable five-membered ring with dimercaprol, there is no evidence that lead acts in a similar manner. Indeed, the greater size of the atom of lead, and the difference in valency possibly indicate that a six-membered ring might prove more stable.

It was with these considerations in mind that experiments were carried out to test the ability of a 1:3- and a 1:4-dithiol to promote the excretion of lead. From the limited information available, it appears that lead combines more readily with a 1:3-dithiol than with either a 1:2- or a 1:4-dithiol; at any rate, the urinary excretion of lead was greatest after the administration of 1:3-dimercaptopropanol, in spite of the small dose of this substance which was given.

Subacute distributions:

The distribution of lead in the untreated animal showed no unexpected features. As has generally been found in rabbits (Kisskalt and Friedmann, 1914; Lomholt, 1924; Kehoe and Thamann, 1933) the excretion/

excretion of lead was greater in the faeces than in the urine after the first day or two, and the combined excretion amounted to about half the total dose in three weeks. The rate of excretion roughly followed an exponential curve, and has reached a very low level by the end of the experiments.

The lead remaining in the body was located mainly in the bones; bone marrow and liver were the only other tissues in which concentrations usually exceeded 1µg. of lead per gramme. The present figures for the concentrations of lead in skeletal muscle and brain are lower than those obtained by Kisskalt and Friedmann (1914) and by Kehoe and Thamann (1933). However, the method employed by the latter authors was unreliable (Kehoe et al. 1935), and their figures may be disregarded.

The effects of treatment were small. The administration of dimercaprol possibly induced a slight increase in the urinary excretion of lead. Germuth and Eagle (1948) observed larger increments in the urinary excretion of lead by rabbits up to eleven days after the last dose of lead, but they had administered/

administered several doses of 200mg. of lead acetate subcutaneously and their rabbits therefore had a depot from which lead was almost certainly being continuously absorbed and was in addition possibly mobilized by dimercaprol. Even so, as here, successive doses of dimercaprol had a rapidly diminishing effect on urinary excretion, and any effect on faecal excretion was obscured by a diminished output of faeces. The amount of lead in blood cells decreased during treatment, and probably accounted for a large part of all the extra lead excreted. As stated previously, the bones may also have contributed a little. A decreased concentration of lead in the bones of rabbits poisoned with large amounts of lead and treated with dimercaprol has been reported by Braun et al. (1949), but treatment was initiated at an early stage of poisoning. The data of Ryder et al. (1947) and of Telfer (1947) suggest that dimercaprol has rather more effect on the lead of the blood cells, and urine in man than in rabbits. Ryder et al. (1947) also state that extended courses of treatment with dimercaprol were prohibited by the occurrence of toxic effects due to the dithiol/

dithiol. It seems possible that the use of dimercaprol glucoside might overcome this difficulty.

The changes after parathyroid extract were also minimal. There was no increase in the excretion of lead in the blood cells. It does not appear that, with the doses used in the rabbit, parathyroid extract has any appreciable effect on the distribution of lead once most of the lead is deposited in the bones. Consequently, the expectations based on the use of dimercaprol and parathyroid extract together were not fulfilled, and the negative results of the combination must be attributed to the lack of action of the parathyroid extract alone.

Dithiols.

Few generalizations on the actions of dithiols can be made from these studies. The one consistent finding is that the urinary excretion of the poison was increased in each case by treatment with dithiols, while the faecal excretion was not significantly altered. The increased urinary excretion has been found in various studies of the effect of dimercaprol in metallic poisoning - by arsenic (Chance and Levvy, 1947), by zinc (Bruner, 1950), and by cadmium (Tepperman, 1947), though in the latter case the faecal excretion was also enhanced. It has also been reported that dimercaprol glucoside increases the urinary excretion of copper and zinc in normal subjects (McCance and Widdowson, 1946). It appears, therefore, that the antidotal action of the dithiols consists not only of an inactivation of the poison but also of an increased elimination of the substance from the body.

There was no consistent effect by dithiols on the tissue distributions of the elements investigated in the present work. For instance, dimercaprol greatly/

greatly reduced the concentration of mercury in the kidneys, but did not affect the concentrations of gold or lead in that site although those concentrations were quite high; and Tepperman (1947) has reported that the amounts of cadmium in the kidneys of animals poisoned with this metal are increased by treatment with dimercaprol. Dimercaprol glucoside increased the concentration of mercury, but not of lead, in the plasma, while dimercaprol had no effect on the concentrations of either element in the plasma. It appears that at present, the actions of dithiols on the tissue distributions of any metal cannot be predicted from their actions on the distribution of another metal; the effect of each dithiol on each element must receive individual investigation.

The different tissue distributions shown by metallic substances is indicative in itself of the varying affinities of these substances for certain chemical configurations. Mercury does not concentrate in the spleens of rabbits, and is readily removed from the body by treatment with dimercaprol; gold and lead occur in high concentrations in the spleens of these animals and are not readily removed from/

from there, or indeed from the rest of the body, by dimercaprol. Possibly, therefore, a knowledge of the constituent of the splenic cells with which gold and lead combine might indicate another type of compound possessing greater affinity for these elements than does dimercaprol, and hence possessing greater antidotal activity. An alternative, and more empirical, approach to the problem is the testing of other dithiols and compounds known to combine with gold and lead in vitro. As has been shown, 1:3-dimercaptopropanol has given promising results in poisoning with lead. The essential point appears to be that recognition of the chemical affinities of metallic poisons must lead to a specific antidote for each poison.

Summary/

SUMMARY

1. Studies have been made of the distribution and excretion of mercury, gold and lead, in rabbits, without and with treatment with dithiols. The method involves the use of radio-active isotopes of these elements.

2. Mercury.

After the intravenous injection of 5μM./kg. of mercuric chloride, the highest concentrations of mercury were found in the kidneys. Of the other tissues, only the liver contained more than one microgramme of mercury per gramme consistently. The excretion of mercury was slow and accounted for only two-thirds of the dose after two weeks, occurring mainly in the urine.

After treatment with dimercaprol, much smaller amounts of mercury were found in the kidneys. The excretion of mercury in the urine was greatly enhanced, but the faecal excretion was unaffected. These changes were found even when/

when treatment was delayed until nine days after poisoning.

Similar changes occurred after the administration of dimercaprol glucoside, except that the concentrations of mercury in the plasma, and the biliary excretion, increased. The urinary excretion of mercury was less than that obtained after treatment with dimercaprol.

3. Gold.

After the intravenous injection of 0.01mM./kg. of gold chloride, high concentrations of gold were found in the spleen, kidneys, plasma, bone marrow, liver and bile. Excretion occurred very slowly, about one-fifth of the dose being eliminated in the urine and faeces in five days.

After treatment with dimercaprol, the concentrations of gold in the blood were reduced and the urinary and biliary excretion increased. No substantial change occurred in the amounts of gold found in other tissues, or in the faecal excretion.

4. Lead.

High concentrations of lead were found in the/

the liver, spleen, bone marrow, epiphyses, and kidneys, twenty-four hours after the intravenous injection of 0.01mM./kg. of lead acetate. Only 1-4 per cent of the dose was excreted in the urine in this period.

After the administration of dimercaprol glucoside, the quantities of lead in the liver and blood cells decreased. This was accompanied by increased urinary and biliary excretion of lead.

5. Both dimercaprol and dimercaprol glucoside increased the urinary excretion of lead after the injection of 0.1mM./kg. of lead acetate, and were approximately equally effective.

6. After the intravenous injection of 0.01mM./kg. of lead acetate, treatment with 1:4-dithioerythritol slightly, and with 1:3-dimercaptopropanol greatly, increased the urinary excretion of lead.

7. Twenty-one days after the intravenous injection of 0.01mM./kg. of lead acetate, about 50 per cent of the dose had been excreted, predominantly in/

in the faeces, and the bones contained about 25 per cent of the dose. The bone marrow and the liver were the only other tissues which consistently contained more than 1 per cent.

Treatment with dimercaprol or parathyroid extract or both, caused no substantial change in the distribution or the excretion of lead, apart from a transient increase in the urinary excretion after dimercaprol.

8. The method and the significance of these results are discussed.

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