at least one of the doses for assay. The results are given in Figs. 1 and 2. It will be seen that 100,000 units of penicillin given alone produces a satisfactory blood concentration for several hours. With the addition of potassium citrate the peak concentration is raised, but there is little difference in the duration of an appreciable blood concentration. The results of administration of penicillin simultaneously with aluminum hydroxide gave essentially similar results.

Fig. 3 indicates the blood level of penicillin of a patient who received 100,000 units intravenously.[6]

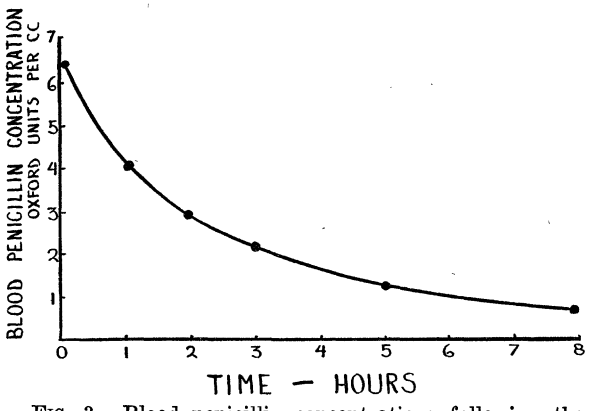

FIG. 3. Blood penicillin concentrations following the intravenous injection of 100,000 units into a patient with complete anuria.

This white female, 46 years of age, had 3 days previously taken a lethal dose of mercury bichloride. At the time the penicillin was injected a complete anuria existed so that no urinary excretion was possible. The blood concentration, however, decreased at a rate which suggested that the half life of penicillin in the body was approximately 2 hours. This indicates that penicillin is destroyed, inactivated or excreted by some route in addition to the kidneys. This disappearance of penicillin in the body proper is also indicated by the fact that only 60 per cent. of intramuscularly administered penicillin is excreted. It has also been noted when the urinary excretion rate is decreased by diodrast[7] or para-amino hippuric acid[8] that the total amount of excreted penicillin decreases from approximately 60 per cent. to 30 per cent.

The destruction of penicillin in the body proper may be the basis for the difference in the excretion of penicillin when administered orally or intraduodenally as compared with the excretion of parenterally administered penicillin. In the one instance the excretion is exceedingly rapid during the first few minutes after injection so that the exposure time to "destructive" influences is quite short. On the other hand, penicillin absorption from the gastrointestinal tract results in a more uniform rate of entry of penicillin into the circulation and in general the rate of excretion is fairly uniform during the first 2 hours after ingestion. This results in a longer exposure of penicillin to possible destructive influences. It may be significant that orally or duodenally administered penicillin enters the portal circulation and is exposed to the metabolic activities of the liver, whereas parenterally administered penicillin is not nearly so completely exposed to this influence. It is also known that certain of the bacteria of the intestinal flora elaborate penicillinases which rapidly inactivate penicillin. However, the site of absorption of penicillin and the location of significant amounts of the enzyme in the intestine are not sufficiently defined to indicate whether or not such destruction may be of significance.

[6] The studies on this patient were done in collaboration with Dr. Max. Miller, of the Department of Medicine, School of Medicine, Western Reserve University, and the Medical Service of University Hospitals, Cleveland, Ohio.

[7] C. H. Rammelkamp and S. E. Bradley, *Proc. Soc. Exp. Biol. and Med.*, 53: 30, 1943.

[8] K. H. Beyer, L. Peters, R. Woodward and W. F. Verwey, *Jour. Pharm. and Exp. Therap.*, 82: 310, 1944.

SUMMARY

A comparison of penicillin excretion when equivalent quantities of the drug are given orally or parenterally indicates that approximately 60 per cent. urinary excretion occurs after parenteral administration, whereas 14 per cent. urinary excretion occurs following oral ingestion. That destruction by gastric acidity is not primarily responsible for this difference is indicated by the fact that administration of penicillin directly into the duodenum does not greatly alter the amount of penicillin excretion. Evidence indicating that the majority of orally administered penicillin is destroyed by the body proper is discussed.

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IMPAIRMENT OF REPRODUCTION IN RATS BY INGESTION OF LEAD

ACCIDENTAL contamination with lead of an experimental diet for rats led to results which appear confirmatory of the thesis that sterility, high incidence of abortion and excessive infant mortality in human populations may be caused by chronic lead poisoning.[1] Report of our results is of interest because Calvery and associates in an extended series of papers[2] were unable to reproduce such effects of lead poisoning either in rats or dogs by feeding lead acetate or lead arsenate. It may be significant that our animals ingested lead largely in metal form.

[1] R. M. Hutton, "Lead Poisoning; a compilation of present knowledge." Provincial Board of Health of Ontario, Toronto. 1923.

[2] *Jour. Pharmacol.*, 64: 364–464, 1938.

A total of 78 pairs of rats and their progeny were involved in our study. The experimental diet consisted of an intimate mixture of about 65 articles of food which are most prominent in the American diet, in the approximate proportions in which they are consumed. This diet was inaugurated in late January, 1941, during the late stages of the pregnancies which gave rise to the litters from which these 78 pairs were selected and was continued through a period of about nine months. Growth of the original young was normal and no suspicions arose until the progeny of these young began to appear. The mortality among the young of the second generation was very high, ranging from 20 to 80 per cent. and averaging about 50 per cent. for the entire period. Most of the deaths occurred at birth or within a day or two thereafter. Many young were destroyed by the mothers as they approached weaning age. The young which survived were without exception stunted in growth, attaining weights of from 30 to 45 grams at 28 days of age. They appeared anemic and their fur was stained brown, and was greasy and matted.

A number of maternal deaths occurred near the end of term. In addition, some five males and seven females became quite sterile. Two of these females recovered fertility after transfer to the Sherman diet (one-third whole milk, two-thirds whole wheat) for ten to twelve weeks. Several other mothers also later reared normal young after transfer to this diet.

From time to time throughout their adult lives, individuals of the original 78 pairs contracted what appeared to be pulmonary disease. A total of thirteen rats died from this cause. Of these, seven were thoroughly examined post mortem and all found to have died of pneumonia which in several cases was lobar in distribution and croupous in character. A type XIX pneumococcus was recovered from three of the seven. Since many were examined long after death, the actual incidence may have been higher. It was further noted that the kidneys were of a dark red color and apparently somewhat enlarged. In the histologic preparations large, acidophilic inclusion bodies were found to be numerous in the epithelium of the convoluted tubules of every animal (Fig. 1). Casts and thickened blood vessels were seen in some cases as well. Seven other rats were therefore sacrificed. None of these had pneumonia, but inclusion bodies were again present in every animal. It was evident that, while the deaths were due to pneumonia, all the animals suffered from another, unrelated disease.

Identical inclusion bodies have been described by Blackman[3] in the kidneys of infants poisoned by lead and in rats fed lead acetate. Accordingly, seven lots of the ration were assayed for lead. The lead content varied from 0.5 to 12 mgs per 100 grams, the average value being 3.5 mgs. The source of the lead was found to be certain concealed babbitted bearings of the masticator used in mixing the ration. The

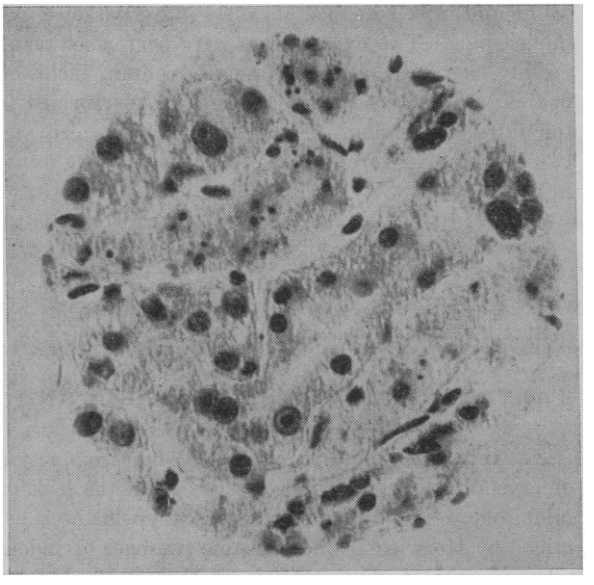

FIG. 1. Rat kidney, showing acidophilic, intranuclear inclusion bodies, abnormally large nuclei and black, granular deposits.

food did not come in direct contact with these bearings, but small variable amounts of lubricating grease, heavily charged with lead, exuded occasionally through crevices at the ends of the blades into the mixing chamber.

In addition, the kidneys and livers of seven rats were examined for lead by the dithizone method. Five of these had been fed the mixed diet continuously for seven months. All five had many inclusion bodies. The lead values ranged between 0.25 and 0.7 mgs per 100 grams fresh tissue. The sixth rat had been fed Sherman's diet for 11 weeks after about 6 months on the leaded diet. The lead content of its kidney and liver tissues was 0.024 mgs per 100 grams. The seventh animal, after six months on the leaded mixed ration, was given the Sherman diet for three weeks before it was sacrificed. The lead value in this case was 0.18 mgs. In neither of the latter two animals were inclusions found.

Eighteen rats less than three weeks old were also examined. These varied from newborn to animals about to be weaned. In none were inclusion bodies found. Three litters were examined for lead, using pooled kidneys and livers. In every case the amount of lead present was less than in the mother. In two

[3] S. S. Blackman, Jr., *Bull. Johns Hopkins Hospital*, 58: 384, 1936.

cases, the values were approximately a third less and in one case less than a half.

The relationship, if any, of the incidence of pneumonia in the adults to lead poisoning is unknown.

In view of Hindle's[4] report, an attempt was made to transmit the inclusions to animals fed standard diets. Kidney suspensions were prepared in broth-saline and injected subcutaneously into rats, mice and guinea pigs. Two experiments were performed using freshly harvested kidneys known to contain inclusion bodies. In one case blind passage was performed to the third generation. Ten rats, fourteen mice and two guinea pigs were injected. Inclusions were found in none.

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ANTAGONISM BETWEEN HEPARIN AND PLASMA TRYPSIN

HEPARIN was found to inhibit the digestive action of crystalline trypsin upon casein[1, 2] and the lack of inhibitory action upon commercial trypsin[3] was ascribed by Horwitt[4] to the probable presence of indefinite amounts of chymotrypsin in those preparations to be found in the market, since chymotrypsin is not inhibited by heparin. On the other hand, heparin is being largely used as therapeutic agent in thrombosis and recently[5] it was reported the protective action of heparin against necrosis produced by extreme local cold (frost bite). Since activation of plasma trypsin might constitute a common mediator in many manifestations following thrombosis and platelet disintegration, we have found it advisable to study the effect of heparin upon the proteolytic enzyme found in normal plasma. Trypsin is present in plasma in a free (I) and a bound (II) condition[6,7] and can be estimated following precipitation either with acetone (I) or with a 2.5 per cent. solution of trichloracetic acid (II), incubation of the whole precipitate (resuspended in buffer pH 8.4) for 48 hours and final estimation of the N P N. Heparin added either before precipitation or after the preparation was set up for incubation, had a strong inhibitory effect as shown in Table 1.

TABLE 1

Exp. No.	Material used	Amount of heparin* added	Trypsin (mgm NPN/100 ml plasma) Total	Free
I	(a) dogs plasma	0	91.4	31.9
	(b) same + heparin	2 mgs	85.6	3.5
	(c) same + heparin	5 mgs	34.8	−1.0
II	(a) dogs plasma	0	114.0	19.4
	(b) same + heparin	10 mgs	12.6	4.8
	(c) same + heparin	10 mgs	49.1	...

* The heparin used in those experiments was a crystalline-sodium salf of beef heparin (11 units per mgm) kindly supplied by Dr. L. B. Jaques of Toronto, Canada.
Note: In experiment II c, heparin was added after precipitation by trichloracetic acid and immediately before incubation.

The fact that heparin displays a definite inhibitory effect upon plasma trypsin when added before activation of the enzyme by addition of trichloracetic acid might be explained by assuming that it strengthens the effect of the natural inhibitor present in plasma. This agrees with Ferguson's view[7] that the polypeptide-like inhibitor of trypsin present in plasma might have acidic groups analogous to those of heparin or that heparin might constitute a prosthetic group for this inhibitor. A more extensive report will follow this note.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE USE OF A PLANIMETER IN VOLUME STUDIES OF EARLY EMBRYOS

THE shape of developing ova after the first cleavage division does not allow accurate volume calculation on the basis of diameter or radius, a fact which must at least partly explain the absence of data pertaining to this problem.

The volumes of more than eighty ova and blastocysts have been successfully ascertained by the planimetry of serial sections of known thickness.

Serial ten micron sections of the specimens were projected at two hundred diameters of magnification and the outline of each section was accurately traced on suitable paper. The average of ten planimeter readings was taken for each section and the values

[4] E. Hindle, *Nature*, 129: 796, 1932. E. Hindle and F. Coutelen, *Compt. Rend. Soc. Biol.* 111: 870, 1932.
[1] M. K. Horwitt, SCIENCE, 92: 89, 1940.
[2] A. J. Glazco and J. H. Ferguson, *Proc. Soc. Exp. Biol. and Med.*, 45: 43, 1940.
[3] J. A. Wells, C. A. Dragstedt, J. A. Cooper and H. C. Morris, *Proc. Soc. Exp. Biol. and Med.*, 58: 57, 1945.
[4] K. Lange, L. J. Boyd and L. Loewe, SCIENCE, 102: 151, 1945.
[5] A. Schmitz, *Z. physiol. Chem.*, 250: 37, 1937.
[6] N. K. Iyengar, K. B. Sehra and B. Mukerji, *Ind. med. Gaz.*, 57: 348, 1942.
[7] J. H. Ferguson, SCIENCE, 97: 319, 1943.