lesser extent, depending on the biological system involved, and may require special conditions.

We have also carried out some experiments on the fermentation of pyruvic acid by the propionic acid bacteria. During this fermentation carried out in the presence of C^*O_2 a relatively large amount of the fixed C^* was found in the form of a carbonyl compound, precipitable as a 2,4 dinitrophenylhydrazone along with pyruvic and oxalacetic acid present as carriers. The possibility of this compound being an α-keto acid with C^* in the carboxyl group is strongly suggested by the enzymatic decarboxylation of the radioactive compound with yeast carboxylase, in which C^*O_2 was obtained. Radioactive propionic and succinic acids were also formed in this fermentation. Details of these and other experiments will be published elsewhere.

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S. F. CARSON
J. W. FOSTER[7]
S. RUBEN
M. D. KAMEN

HOPKINS MARINE STATION OF
STANFORD UNIVERSITY,
DEPARTMENT OF CHEMISTRY AND
RADIATION LABORATORY OF THE UNIVERSITY
OF CALIFORNIA AT BERKELEY

MASSIVE "ACUTE" PRECIPITATION OF FREE SULFATHIAZOLE IN THE URINARY TRACT*

THE formation of concrements in the urinary tract after chronic administration of sulfanilamide derivatives has been reported repeatedly in the literature.[1, 2, 3] The uroliths were always found to contain a high percentage of the very insoluble *acetylated* form of the different compounds. After administration of sulfapyridine or sulfamethylthiazole the concrements were located mainly in the renal pelves, ureters and bladder, whereas, after sulfathiazole administration, considerable intrarenal precipitation (in the collecting tubules) was observed.[4]

In the course of an investigation on the acute intraperitoneal toxicity, in rats and mice, of the 3 derivatives[5] mentioned above, the peculiar observation was made that all animals dying from a single dose of sulfathiazole or its sodium salt, without exception, showed massive precipitation of the *free* drug in the urinary tract. Depending upon the dose, and consequently upon the time-interval between injection and death of the animal, the precipitate was found in different parts of the urinary tract. If death occurred only a few hours after the injection, the collecting tubules and the papillary ducts were filled with a whitish material distinctly visible macroscopically and extending into renal pelves, ureters and bladder. After a longer time-interval, the precipitate in the kidneys diminished in amount and finally disappeared (usually after 24 hours), while the bladder became completely filled and even distended with a white crystalline material, which in some cases reached back into the lower parts of the ureters. Within 10 to 20 hours this soft precipitate in the bladder was converted into hard aggregates weighing between 5 and 30 mg and composed almost entirely of free sulfathiazole. If the animals survived for at least several hours, anatomical signs of irritation were often found in the kidneys (marked enlargement with congestion and edema).

The picture as described in the different stages was seen in 54 rats and 20 mice injected with various doses of sulfathiazole or its sodium salt.

This phenomenon of acute precipitation was further investigated by sacrificing groups of 3 rats at different time-intervals after the intraperitoneal injection of a sublethal dose of sodium sulfathiazole (1.0 g/kg). The urinary tract was examined carefully and drug determinations in blood and various tissues were performed. Some of the results are summarized in Table I.

TABLE I

DETERMINATION OF SULFATHIAZOLE

Time after injection	Blood		Kidney				
	mg. per cent.		mg. per cent. per moist tissue		Total amount in both kidneys in mg.		Gross precipitation in the renal papilla
	Free	Acetyl.	Free	Acetyl.	Free	Acetyl.	
5 min.	103	0	145	0	2.12	0	0
15 min.	101	0	259	0	4.32	0	±
30 min.	115	0	396	0	6.06	0	++++ massive
60 min.	107	0	322	0	5.06	0	+++
3 hrs.	58	0	266	25	4.96	0.46	++
12 hrs.	44	7	67	27	1.76	0.70	+
24 hrs.	10	5	17	9	0.32	0.18	0

Intraperitoneal injection of sodium sulfathiazole 1.0 g/kg in rats. All figures are the mean of the values from 3 animals.

It can be seen from the table that precipitation (composed entirely of *free* sulfathiazole) reaches its maximum in the renal papilla 30 minutes after the

[7] National Research Council Fellow.

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[2] P. Gross, F. B. Cooper and M. Lewis, *Proc. Soc. Exper. Biol. and Med.*, 40: 448, 1939.

[3] H.Molitor and H. Robinson, *Proc. Soc. Exper. Biol. and Med.*, 41: 409, 1939; *Arch. internat. de pharmacodyn. et de therap.*, 62: 281, 1939.

[4] P. Gross, F. B. Cooper and R. E. Scott, *Urol. and Cutan. Review*, 44: 205, 1940.

[5] D. Lehr, W. Antopol, J. Churg and H. Sprinz, *Proc. Soc. Exper. Biol. and Med.* In press.

intraperitoneal administration and disappears completely within 24 hours. Acetylation becomes apparent at about 3 hours after the injection. The deposition of relatively increasing amounts of the extremely insoluble acetylated derivative may be responsible for the renal irritation.

The data presented demonstrate the extreme rapidity of absorption and excretion of sulfathiazole and lend further support to the view which has been expressed elsewhere: that the acute precipitation in the urinary tract is due mainly to a high rate of elimination of sulfathiazole from the body. Thus after concentration of the glomerular filtrate by reabsorption of water, precipitation occurs in the collecting tubules.

A similar picture could not be produced with sulfanilamide, sulfapyridine, sulfamethylthiazole or their sodium salts.

DAVID LEHR
WILLIAM ANTOPOL
JACOB CHURG
THE NEWARK BETH ISRAEL HOSPITAL,
NEW JERSEY

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A MODIFICATION OF RIDDLE'S METHOD OF PROLACTIN ASSAY

WHILE we were comparing the alkaline alcoholic extraction method of Bates and Riddle[1] with the acid acetone extraction method of Lyons[2] for preparing lactogenic hormone of the anterior pituitary, it became necessary to use a large number of pigeons for accurate assaying of various fractions used in the investigations.[3] The purchasing and keeping of the pigeons, however, became so expensive that a way had to be found to reduce the cost as much as possible. The method described below is the result of such an attempt and enables one to assay the hormone without sacrificing the birds. Thus the cost of investigation was greatly reduced.

As in the original method of Riddle, Bates and Dykeshorn,[4] we used both male and female pigeons. On the previous day, before the injection of the sample, the feathers on the pectoral regions of the birds as well as on the area around the crop sac on the neck are carefully plucked. Before the injection of the sample, the skin layer over the crop sac on either right or left side of the median line is incised to the length of about two centimeters, so that the crop sac can be clearly seen. The crop sac is usually colorless and transparent and the presence of food material such as wheat, oats and barley within the sac is clearly discernible. If the crop sac is opaque and thick as in the nesting and nursing period, the pigeon is not suitable for the assay of the hormone and should be exchanged for another bird. Only the pigeons with clear transparent sac are used for the assay. After examination of the sac, the opening is closed by sewing with surgical suture and an appropriate antiseptic, such as tincture of iodine or merthiolate solution, is applied. As in the original method, a definite quantity of the sample solution is injected once daily for four days. On the fifth day (ninety-six hours after the first injection) the crop sac located on the other side of the one previously examined is exposed and examined. According to the condition of the thickening of the sac membrane, the potency of the hormone solution is evaluated.

The method is easy to execute with a little practice. It should be borne in mind, however, that the examination of the sac should be made as soon as it is exposed. The irritating operations such as rubbing of the exposed area with alcohol-soaked cotton swabs or cheesecloth must be avoided, since a slight irritation of the crop sac membrane will result in the thickening of the membrane and may cause an erroneous evaluation of the potency of the sample. It is important to note that during the incising of the skin layer over the sac, great care should be exercised to avoid tearing the crop sac membrane. If, however, the crop sac membrane is cut, the area should be immediately closed by holding together the surrounding membrane and tying with sterile silk thread.

After the examination of the sac, the incised skin layer is closed by sewing with surgical suture and an antiseptic is applied. The pigeon is then kept in a large cage for three to four weeks or until the incised area is perfectly healed. They are then ready to be used again for prolactin assay.

The authors are aware of the fact that an accurate assaying of the hormone by this method or any other biological method is a difficult task. This is due to the variation in the sensitivity of the individual birds, even among the same strain.[1,2] The difficulty can be overcome if a large number of birds are used for the assay of a sample and also by using a standard preparation for comparison as recommended by Bates and Riddle or by the Commission on Biological Standardization of the League of Nations. The authors believe that by the use of the method described here and with

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