as substrate. The results ran parallel with those obtained in the perfused hind legs of the frog. The oxygen consumption was completely inhibited by HCN but not affected by octyl alcohol.

Discussion. The inhibitory action of amine-oxidase and tyrosinase on the product of the interaction between renin and hypertensinogen constitutes an indirect proof that the vasoconstrictor substance is a phenolic pressor amine as has been suggested by Holtz, Bing and Schroeder. Even though the enzymes used in these experiments have not been completely purified up to the present, their different behavior towards HCN, octyl alcohol, etc., is in agreement with the conception that the activity of squid extracts is due to amine-oxidase and that of the mushroom extracts to tyrosinase.

Summary. The vasoconstrictor substance (hypertensin, angiotonin) obtained by the interaction of renin and hypertensinogen and tested on the Loewen-Trendelenburg preparation is destroyed (enzymatic destruction) in contact with amine-oxidase (extract of *Sepia officinalis* liver) or tyrosinase (extract of *Psalliota campestris* liver).

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Sex and Proteinuria of Mice.*

L. F. WICKS. (Introduced by E. V. Cowdry.)

From the Research Department, Barnard Free Skin and Cancer Hospital, St. Louis, Mo.

While engaged in an investigation of possible systemic effects of methylcholanthrene on mice, many samples of their urine were analyzed. These urines frequently contained a surprisingly large amount of coagulable protein. This was regarded as due to the toxicity of the hydrocarbon until examination of normal, untreated and apparently healthy animals also revealed a high proteinuria. Further observations disclosed that this proteinuria is essentially sex-linked, for it was encountered very conspicuously in the males and usually only to a much smaller degree in females. The urine samples were from 9 pure strains (Swiss, A, New Buffalo, Old Buffalo, D, C_3H, CBA, AKA, C57 Black) and mixed stock mice. The mixed stock animals were of vague ancestry but definitely no

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TABLE I.

Proteinuria Differences in Groups of Male and Female Mice of Various Ages and Strains.

♂		♀	
Strain A.			
¾	++	1	tr.
1½	+	1¼	0
2	+++	1½	0
3	++++	1¾	f. tr.
3-4	+++	2-4	tr. to +
4	near. ++++	3-4	tr.
5½	+++	4	f. tr.
6	++++	4¾	tr.
*6+	+++	*6+	f. tr.
6+ d.g.	+++	10	tr.
8	+++	†10	+
10	++++	19 (1 mouse)	0
Old Buffalo.			
¾-1	+ to ++	1	tr.
1¼	+	1¼	f. tr.
1¾	+++ to ++++	1¾	0
1-2	+++	1¾ d.g.	tr.
2	++	1-2	++
2½	+++	*2-3	f. tr.
2, 3	++ to +++	3-4	+
3	+++ to ++++	7-8	tr.
3½	near. +++	8-10	+ to ++
4	+++	10-12	+
4,5	++ to +++		
8 (1 mouse)	+++		
AKA.			
9-10	+++	9-10	0
Same 9½-10½	+++ or more	Same 9½-10½	0
CBA.			
4-5	++++	3-4	++
Same 4½-5½	++	Same 3½-4½	0
5½-6½	++++	4-5	less than +
Mixed Stock.			
1¼ K-C	+++	1	0
1¼	+	1¼	+ or less
1½ K-C	near. +++	1½ K-C	tr. to +
1¾ K-C	++++	3-3½	f. tr.
1¼ K-C, d.g.	+++	3½-4¼	tr.
2 K-C	++	Same group diff. mice	+
3	+++	6	tr.
3-3¼	++++	7	+ or less
3-4	+++	Indefinite	f. tr.
4	++++	*Several (?)	0
Several (?)	+++		
Indefinite	+++		
6+	+		
Indefinite	near. +++		

New Buffalo

♂		♀	
1	+ to ++	1	tr.
1½	tr.	1 d.g.	tr.
2	++++	2	+ or more
2½	+++	2 d.g.	tr.
2½ d.g.	+++	2½	++
2¾	++++	3¼	tr.
3	+++ to ++++	5	tr.
3¼	+++	6½	0
5	++++	7	tr.
7	++++	*8	0
*8	++ to +++		

Swiss.

¾-1	++ to +++	¾-1	0
1½-2	near. ++	2¼	tr.
3	++ to +++	2-3	tr.
4	++++	4	tr.
4 d.g.	++++	4 d.g.	tr. to +
4 d.g.	+++	5	0
5	+++	6+	near. ++
6	++++	7	tr.
7	+++ or more	8	tr. to +
7-8	+++ or more	9-12	tr.
*10-12	+++		

D.

3	+++ to ++++	3-4	++
Same 3½	+++	Same 4-5	+
6	++ to +++	6	tr.
10	++ to +++	10	f. tr.
15	near. ++++	15	tr.

C57 Black.

2-4	++	2-4	0
4-5	near. ++++	4-5	++ or over
Same 5-6	++++	Same 5-6	near. +++
2-3	++	2-3	f. tr.
Same 3-4	++++	Same 3-4	tr.
Repeat	++++	Over 12	tr.

C_3H.

5½-6	+++	5-6	++
Repeat	+++	Repeat	near. +++
9	++++	2nd repeat	++ to +++
Repeat	+++	9-10	tr. or over
9-9½	+++ or over	Repeat	++
Repeat	+++	2nd repeat	near. ++
		12	tr. to +
		Repeat	++
		2nd repeat	near. ++

*Unusually large volume.
†On high protein diet for 8 months.
0—No test with T.C.A.
f. tr.—Faint trace, just visible turbidity.
tr.—Trace, cloud only, no ppt.
d.g.—Different group of animals.

longer of pure line. (Those designated "K-C" were a cross between "mixed stock" and a hairless strain.) The C_3H mice exhibited less sex difference and more irregularity than any other strain examined. The results were otherwise quite generally consistent throughout, proteinuria being detected to considerable extent in males from 3 weeks of age onward, and in greatly reduced intensity in females, even when quite old.

The urine was collected in specially constructed cages, remotely similar to the pattern of Levine and Smith[1] for rats. In each, a double screen bottom covered a wax-lined funnel which led into a test tube containing a little toluol. Provision was made so that the drinking water could not drip into the funnel.

Since mice do not readily tolerate fasting, it was necessary to supply them with food while in the collection cages. Besides, fasting mice show a great reduction in urine volume. The food supplied in the collection cages was a mixture of washed casein, starch, salt mixture, cod liver oil and lard, a diet the mice consumed readily. The last named ingredients were in sufficient quantity to form a stiff paste which the animals could not scatter easily. The preparation was also contained in "non-scatter" food cups.[2] Both food and feces were frequently extracted and tested, but neither ever exhibited more than a trace of soluble, coagulable protein. The mice received this unbalanced diet for not more than 24 hours at one time.

The urine samples as represented in Table I were the pooled excretions of 2 to 4 similar animals, except in the case of very young ones where 5 were used. The left columns give the approximate age in months of each group of mice and the right columns the degree of proteinuria. The urines were centrifuged rapidly, and 1 cc of clear fluid was mixed with 5 cc of 15% trichloracetic acid. In these preliminary observations, the relative amounts of precipitate were arbitrarily recorded as "trace" to "four plus". This designation is considerably higher that that in clinical usage. A quantitative method has been devised and numerical data will be reported subsequently along with a description of the procedure.

Upon investigating the chemical nature of the urinary protein, we were surprised to find that while traces of albumin and globulin are present, the greater portion seems to be nucleoprotein. No reference has been met in the literature on the excretion of large amounts of such a protein in the urine of mice, either as a normal or abnormal

[1] Levine, H., and Smith, A. H., *J. Lab. and Clin. Med.*, 1925, **11**, 168.

[2] Hawk, P. E., and Bergeim, O., *Practical Physiol. Chem.*, 1937, 11th Edition, p. 909.

occurrence. There is a series of observations by one worker, Parfentjev,[3, 4, 5] on the presence of a protein in the urine of normal male mice. He believed it to be a chondromucoid substance and adhered to this view when he later isolated the material in collaboration with Perlzweig.[5] Our analyses indicate that it is a nucleoprotein rather than a mucoprotein. Cold acidification of the urine with acetic acid causes a slow separation of the substance which may then be redissolved in very dilute alkali and reprecipitated. The solutions resulting from many acid hydrolyses of the protein failed to give any reaction for reducing sugar, although the Molisch carbohydrate test was always positive, and hydrochloric acid hydrolysis yielded no evidence of sulphate. Kiliani's desoxy-carbohydrate reaction was obtained as well as the crude aniline acetate test for pentose. Phosphate was always found, and the ammoniacal silver nitrate test for purines proved positive. More complete details may be reported elsewhere.

To exclude the possibility that this urinary protein is of extrarenal origin, and where found in males might be due to contamination with spermatic fluid, urine was drawn from the bladder of mice killed immediately before collection. This revealed a protein content as high as that of voided urine.

The consideration that the urinary protein seems a nucleoprotein makes it unlikely that its presence in urine is due to a renal defect. There are, however, two observations recorded in the literature which made it necessary to test this possibility. Gorer[6] has described extensive degenerative lesions in the kidneys of very old mice of both sexes belonging to A, C57 and CBA strains. Preliminary histological examinations of the kidneys of our younger mice revealed only occasional minor lesions. Griffith and Wade[7, 8] have shown that on certain diets poor in choline, degenerative kidney changes may develop in rats. While our diet was not likely to be so deficient, experiments were carried out in which a daily supplement of 0.5 mg of choline hydrochloride was injected for a period of 3 months. This supplement did not diminish the excretion of urinary protein. The excretion was, however, found to be affected by the protein content of the diet. One containing 33% of casein

[3] Parfentjev, I. A., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1285.

[4] Parfentjev, I. A., *Ibid.*, 1933, **30**, 1064.

[5] Parfentjev, I. A., and Perlzweig, W. A., *J. Biol. Chem.*, 1933, **100**, 551.

[6] Gorer, P. A., *J. Path. and Bact.*, 1940, **50**, 25.

[7] Griffith, W. H., and Wade, N. J., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 188.

[8] Griffith, W. H., and Wade, N. J., *Ibid.*, 1939, **41**, 333.

increased the proteinuria, while a diet with only 5% casein diminished it markedly.

Since the proteinuria in mice is associated with sex, the effect of castrating young male mice was studied. The degree of proteinuria remained unchanged for many weeks following the operation, but was greatly decreased after about 3 months. Addis[9] has reported a similar result for albuminuria of male rats. Bell[10] noticed the appearance of albuminuria as young male rats reached maturity. Our rats show no excretion of protein, and we have found very little mention of any marked proteinuria of these animals.

In connection with the apparent sex-linked character of the proteinuria of mice, the recent report of Crabtree[11] is of great interest. She observed differences in structure of Bowman's capsule between most male and female mice. She cites the experiments of Selye,[12] who noticed that treatment of female mice with testosterone propionate may change the parietal lamina of Bowman's capsule.

Summary. A protein with chemical characteristics of a nucleoprotein was found to be excreted in large amounts by male mice of various ages and strains. Female mice excrete much less protein, frequently none at all. A table is appended.

For the castration of animals and the examination of sections, I am much indebted to Dr. William Cramer.

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Prolongation of Estrus by Injection of Suspensions of Estrogen Crystals.

R. K. RICHARDS, H. C. SPRUTH AND M. K. RUSSELL.

From the Abbott Laboratories, North Chicago, Ill.

The influence of the method of administration upon the duration of the effect of estrogenic compounds has early been recognized

9 Addis, T., *Proc. California Acad. Med.*, 1931-32, 38, and personal communications.

10 Bell, M. E., *J. Physiol.* (Brit.), 1933, **79**, 191.

11 Crabtree, C., *Science*, 1940, **91**, 299.

12 Selye, H., *J. Urol.*, 1939, **42**, 637.