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SECTION MEETINGS

CLEVELAND	
Western Reserve University	February 9, 1940
ILLINOIS	
Northwestern University	January 23, 1940
MISSOURI	
St. Louis University	February 14, 1940
NEW YORK	
New York University	February 28, 1940

11152 P

Preparation of Pyrogen-Free Urogastrone.*

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(Introduced by A. C. Ivy.)

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We have previously reported[1] that a substance which inhibits gastric secretion can be extracted from urine. The nature of this inhibitory factor is unknown, but the possibility was considered that it might represent excreted enterogastrone. Early in the course of experiments designed to test this possibility, it was discovered that the urinary extracts exerted a definite pyrogenic action. Since Meyer,

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1 Culmer, C. U., Atkinson, A. J., and Ivy, A. C., *Endocrinology*, 1939, **24**, 631; Gray, J. S., Wieczorowski, E., and Ivy, A. C., *Science*, 1939, **89**, 489; *Am. J. Physiol.*, 1939, **126**, 507.

Cohen, and Carlson[2] have shown that artificial fever of a severe degree may practically abolish gastric secretion, it became essential to remove the pyrogen from the urinary extracts. It is the purpose of this report to show that extracts of normal urine may contain pyrogen and that the pyrogen is not identical with the gastric inhibitory factor.

Methods. Extracts of the gastric inhibitory factor were assayed in 6 Heidenhain pouch dogs injected subcutaneously with histamine. The potencies of the extracts are expressed as the percentage inhibition of free acid secretion. Rectal temperatures were taken at ½ hourly intervals for a period of 3 or 4 hours after injecting the extract. Pyrogen assays were also made by recording rectal temperature curves in a group of 5 rabbits. The extracts of normal male urine were all prepared by the benzoic acid adsorption method of Katzman and Doisy.[3]

Results. Crude extracts prepared by the original method (Method A) when injected intravenously into rabbits in doses equivalent to 30 cc of urine produced an average rise in temperature of 4.0°F. By thoroughly washing the benzoic acid precipitate in order to completely remove the mother-liquor, *i. e.*, the original urine (Method B), the average rise in temperature was reduced to 2.1°F. Additional precautions consisting of preservation of the urine with tricresol and completing the extraction process in several hours instead of several days (Method C), reduced the average temperature response to 1.0°F.

Attempts were then made to remove the remaining pyrogen by purification procedures. The results are presented in Table I. A fraction, soluble in 50% and insoluble in 75 or 87% acetone, which was obtained from crude material prepared by Method A, was found to contain considerable amounts of both pyrogen and gastric inhibitory factor. A similar fraction obtained from crude material prepared by Method B, contained only traces of pyrogen, but more gastric inhibitory factor than the above. A fraction, soluble in 70% acid (approximately N/10 HCl) acetone and insoluble in 95% acetone, which was obtained from crude material prepared by Method C, contained the gastric inhibitory factor uncontaminated with pyrogen. The substitution of alcohol (95%) for acetone in the latter procedure also yielded a pyrogen-free material, but considerable inhibitory activity was lost or destroyed with this reagent.

Discussion. The results indicate quite clearly that the gastric in-

[2] Meyer, J., Cohen, S. J., and Carlson, A. J., *Arch. Int. Med.*, 1918, **21**, 354.

[3] Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1932, **98**, 739.

TABLE I.
Removal of Pyrogen from Urine Extracts.

Method	Purification	No. of Assays	Dose		Avg temp rise, °F	Avg gastric inhibition, %
			mg	Urine equiv., cc		
A	50-75% neutral acetone fractionation	12	3	240	2.8	—56
B	50-87% neutral acetone fractionation, repeated	6	3	660	.5	—25
,,	,, ,, ,, ,, ,,	6	6	1320	.6	—39
,,	,, ,, ,, ,, ,,	6	12	2640	.8	—55
C	70-95% acid acetone fractionation	6	3	435	0	—39
,,	,, ,, ,, ,,	6	6	870	0	—63
,,	70-95% acid alcohol fractionation	6	3	540	0	—26
,,	,, ,, ,, ,,	6	6	1080	0	—33

hibitory factor is separate and distinct from the pyrogenic factor in the extracts of normal male urine. Until such time as the gastric inhibitory factor is definitely identified, we propose to call it urogastrone.

This work is reported at this time in preliminary form because of its significance for other fields of endocrinology. It is well known that many extracts of various organs are unsuitable for intravenous administration because of fever reactions. We have assayed several commercial extracts of urine and found them to contain pyrogen. Pyrogenic substances may, in fact, be expected to occur in all extracts of biological origin which have not been treated in such a way as either to remove it or to prevent its development.[4] As long as there is no relationship between the effects of pyrogen and those of the specific component of an extract, the presence of pyrogen presents merely a problem of purification. However, when the properties of an extract resemble those of pyrogen, one should be cautious in assuming the presence of a specific component until the absence of pyrogen has been demonstrated. In view of this fact, the testing of extracts of the specific metabolic principle of the pituitary and of the antidiuretic principle of urine for pyrogen would seem to be desirable.

Conclusions. 1. Extracts of normal male urine prepared by the benzoic acid adsorption method of Katzman and Doisy contain large amounts of a pyrogenic substance, as well as the gastric inhibitory factor, urogastrone. 2. Methods for the preparaation of pyrogen-free urogastrone have been developed.

11153

Concentration and Detection of a Dye in Abscesses.

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In a previous communication from this laboratory by Strauss, Neuwelt, Rovner and Necheles[1] a method was described for the detection of hidden abscesses and carcinomas. This method was based on the fact that certain dyes and colloidal substances are concentrated

[4] Ivy, A. C., and Gray, J. S., *Int. Abstr. Surg.*, 1939, **69**, 409.

[1] Strauss, S. F., Neuwelt, F., Rovner, L., and Necheles, H., *Surgery*, 1938, **4**, 930.