ERRATUM.

Article 7032, fourth sentence should read, "The use of autogenous staphylococcus vaccines and the usual sanitary precautions had *during the previous year* failed to keep the disease in check. . . . "

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Hyperglycemia Produced by Extracts of Normal Urine.

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Application of the tungstic acid precipitation[1] to the concentration of the active principles of urine led to the discovery that an extract of the urine of men produces a distinct hyperglycemia in fed rabbits. A review of the literature revealed only the papers of Böhm[2] and Eidelsberg,[3] who reported that the intravenous injection of gonadotropic extracts of pregnancy urine produced a marked hyperglycemia. Dingemanse and Kober[4] and Houssay[5] have added information on this point.

* P represents a preliminary, C a complete manuscript.

[1] Katzman, P. A., and Doisy, E. A., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1188.

[2] Böhm, F., *Z. f. des. ges. exp. Med.*, 1932, **84**, 689.

[3] Eidelsberg, J., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 959.

[4] Dingemanse, E., and Kober, S., *Endocrinol.*, 1933, **17**, 149.

[5] Houssay, B. A., and Biasotti, A., *Compt. rend. Soc. Biol.*, 1933, **113**, 469.

Of course, it is possible that the hyperglycemic action of extracts of urine is due to the diabetogenic substance of the anterior lobe of the hypophysis, (Houssay,[6] Evans,[7] and others) but our work thus far does not permit us to draw such conclusions.

Preparation of Extracts. The substance has been concentrated by the usual methods[1, 8] employed in this laboratory for the preparation of the gonadotropic factor of pregnancy urine. The benzoic acid adsorption process described by Katzman and Doisy[8] gave active extracts of this factor from normal male urine. In this case the acetone insoluble residue, after removal of the benzoic acid, was leached with dilute alkali (pH 8-9) instead of water. Table I presents the data obtained with this type of extract.

TABLE I.
Adsorption Methods.

Method	Preparation No.	Urine equivalent of extract injected Liters	Initial blood sugar Mg %	Maximum blood sugar Mg %	% Increase	Time elapsed before maximum value Hr.
Benzoic Acid	MB 1	12	143	>300	>110	4
	MB 2	4	148	166	12	6
	MB 3	5	117	135	15	4½
	MB 4	10	121	150	24	5
	MB 5	7	145	>300	>107	3
	MB 6	7	136	200	47	3½
Kieselguhr	MA 1A	6	155	153	—	5
	MA 1B	9	163	272	66	4½
Alumina Gel	ML 1	2	143	150	—	4
Kaolin	MK 1	7.6	167	>300	>100	3½
	MK 1	3.8	157	212	35	4
Charcoal	MC 1	3	135	126	—	3½

Tungstic acid precipitation (Katzman and Doisy[1]) was also effective in concentrating the active factor. Clear and more potent extracts are obtained by liberating the active material from the tungstic acid precipitate by means of an alkaloid instead of the sodium hydroxide (Table II). In this procedure the acetone washed tungstic acid precipitate is stirred with an excess of an aqueous suspension of brucine and centrifuged. The brucine is removed by extracting the alkaline solution with chloroform or by precipitation with acetone. Other alkaloids can probably be used in place of the brucine.

[6] Houssay, B. A., and Biasotti, A., *Compt. rend. Soc. Biol.*, 1930, **104**, 407; *Ibid.*, 1930, **105**, 121.

[7] Evans, H. M., Meyer, K., Simpson, M. E., and Reichert, F. L., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 857.

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

TABLE II.
Tungstic Acid Method.

Preparation No.	Urine equivalent of extract injected Liters	Initial blood sugar Mg %	Maximum blood sugar Mg %	% Increase	Time elapsed before maximum value Hrs.
T76b	5-6	137	242	80	—
T103	8	153	245	56	3¼
MR 1	2½	186	311	67	3
MR 2	1.6	139	230	65	—
MR 3	2	129	182	41	6
MR 4*	4	122	150	23	3
MR 5*	4	115	131	14	5
MR 6*	8½	123	146	20	4
MR 7b	14½	127	249	96	4
MR 10	16	137	222	62	5
MR 11	2	168	208	24	4½
MRa 17†	3	165	225	37	3½
MRa 19†	3	161	251	56	3½

*Assayed on animals starved 14 to 18 hours.
†Brucine used for extraction.

Considerable purification and concentration of our extracts can be obtained by acetone precipitation. Addition of 4-6 volumes of acetone does not completely precipitate this factor as the acetone solution is usually somewhat active.

From observations of our crude extracts it appears that the activity is lost on standing in the cold room and on treatment with acid, alkali or heat. The substance is soluble in water, aqueous methyl or ethyl alcohol or acetone and insoluble in ether, absolute alcohol, chloroform and strong acetone.

The extracts were tested for hyperglycemic action by following the blood sugar of rabbits for 5 or 6 hours after subcutaneous injection. Since animals fasted as for insulin assays showed but slight increases in blood sugar, food was not withdrawn until shortly before starting the experiment. This accounts for the high preliminary blood sugar values.