apparent, is increased during the late summer and early autumn, or whether the climatic influence is merely to augment the number of clinically apparent cases without appreciably changing the total number infected.

8753 C

Concentration of a Hyperglycemic Factor from Urine.

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We have previously reported the preparation of a crude urine (male) extract which, when injected into rabbits, causes a hyperglycemia, an increase of acetone bodies and lactic acid in the blood, and a decrease of CO_2-combining power.[1] We now wish to report appreciable progress in the purification of the material responsible for the hyperglycemic effect. We are not as yet prepared to state whether our purified material will respond to the other tests of our earlier (crude) product. The crude substance, in the dry state, can be kept for a considerable time without any marked deterioration. The active material is dialyzable and can be further purified by removing the inorganic sulfates. From approximately 250 liters of urine (male), 52 mg. of a highly active, rather unstable organic substance have been isolated. The amount so far obtained was sufficient for physiological tests (confined to blood sugar determinations) but not for chemical identification.

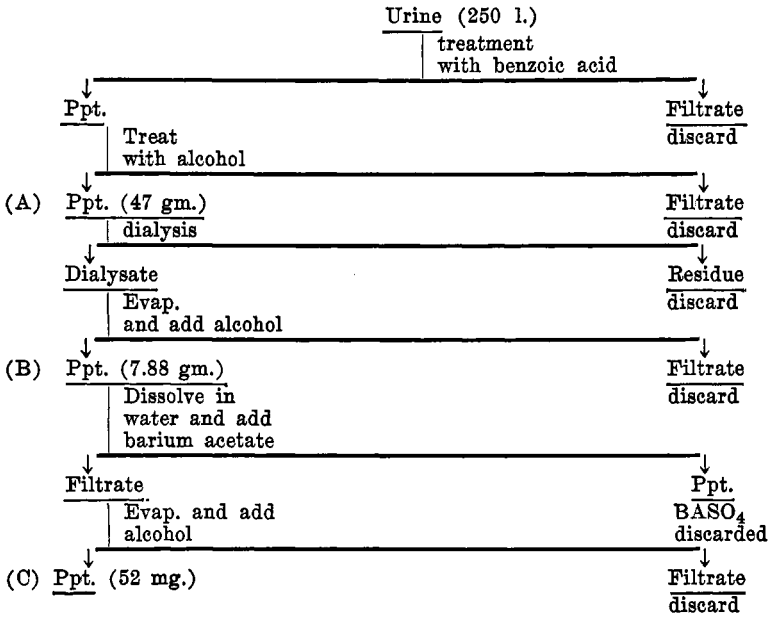
The flow-sheet on the following page illustrates the method of preparing this highly active material.

Since various modifications in the procedures previously described have been adopted, we shall give specific details:

The urine used is obtained from men of 17-22 years of age. It is preserved with thymol and kept in the ice-box. Urine older than 3 days is discarded.

The urine is acidified to methyl red (pH 5) with glacial acetic acid. A warm solution of benzoic acid in alcohol (30 gm. benzoic acid in 60 cc. 95% ethyl alcohol per liter of urine) is added, drop

[1] Harrow, B., *Science*, 1934, **79**, 272; Harrow, B., Naiman, B., Chamelin, I. M., and Mazur, A., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 940: Harrow, B., Chamelin, I. M., and Mazur, A., *Am. J. Physiol.*, 1934, **109**, 436.



by drop, with vigorous (mechanical) stirring. The precipitate is filtered, sucked dry on a Buchner funnel, extracted with alcohol (60 cc. per liter of urine) and centrifuged. (The discarded alcohol-soluble fraction contains benzoic acid, male hormone, etc.) The precipitate is further washed with small portions of alcohol (a total of 30 cc. per liter of urine), twice with ether, centrifuged and dried in a vacuum desiccator. This is fraction (A). This fraction gives positive biuret, Hopkins-Cole and Millons, questionable ninhydrin and Molisch and negative Benedict's. It gives a weak labile sulfur test and a precipitate with phosphotungstic acid. Its activity is equal to 1.1 units per gm. (See Table I.)

We define our unit of activity as equal to that weight (in gm.) of material which, when injected subcutaneously into a 2 kg. female, fed rabbit, produces a 100% increase in blood sugar within the first 3 hours. An average of 3 determinations on different rabbits is taken.

For further purification, (A) is triturated with water (7.5 cc. per gm.) containing a little thymol, and the suspension is dialysed in a cellulose (Visking) membrane against water (15 cc. per gm.). The dialysis is continued for 3 days, the outside solution being replaced with fresh water at the end of each 24-hour period. This dialysis is carried out in the ice-box at 5-10°C.

The combined dialysates are evaporated *in vacuo* to a small volume

TABLE I.

Fraction	Test sample mg.	Glucose	% increase Aver.	Activity per gm., units	Total activity, units
A					
Alcohol-insoluble crude urine extract, 47 gm. from 250 l. urine	500	58.7	56.4	1.1	51.7
	"	60.1			
	"	50.4			
B					
Alcohol-insoluble dialysate 7.88 gm. solids	50	23.0	23.5	4.7	38.6
		24.0			
	"	29.7	28.8	5.8	
		27.8			
	"	21.9	21.0	4.2	
		20.0			
C					
Alcohol-insoluble; free from inorganic salts, 52 mg. solids	2.6	22.3	23.0	88	4.3
		23.7			
	7.5	56.7	55.2	74	
		53.7			
	13.0	123.2	114.7	88	
		106.2			

(keeping the temperature below 37°C.) and alcohol is added to make a 95% solution; it is kept in the ice-box overnight. The material is centrifuged, washed with alcohol and ether and dried in a vacuum desiccator. This is fraction (B). It gives negative protein tests but contains a large percentage of potassium sulfate. Its activity is equal to 4.9 units per gm. (See Table I.)

The next step in the purification is to remove the sulfates. Most of (B) is soluble in water. The $SO_4^=$ in the solution is quantitatively removed with barium acetate, the filtrate, free from $BaSO_4$, is evaporated *in vacuo* to a small volume, and alcohol is added to make a 95% solution; it is kept in the ice-box overnight. The precipitate which forms is centrifuged, washed with alcohol to remove the last traces of potassium acetate, then with ether and dried in a vacuum desiccator. This is fraction (C). Its activity is equal to 83 units per gm. (See Table I.) It appears to be entirely organic in nature and somewhat unstable on drying, turning brown and gummy.

Table I gives a summary of the results.

Summary. 1. We have concentrated our active material from an activity of 1.1 units per gm. to 83 units per gm. 2. Approximately 250 liters of male urine yields 52 mg. of this highly active, somewhat unstable substance.