Great differences in the amount of C^{14} appearing in the tissue protein are also noted when the results from labeled plasma proteins A and B are compared (Fig. 3). This is particularly true when the proteins are given orally. The labeling of some tissues after protein B may be almost twice as high as after protein A.

It is also noteworthy that following the oral administration of either of the C^{14}-labeled proteins the tissue C^{14} is higher than that attained after parenteral administration. In the latter case part of the activity measured in the tissues must be due to C^{14}-labeled plasma and lymph(2,7). With the protein B lower respiratory $C^{14}O_2$ accompanies the higher tissue C^{14} when the dose is given parenterally. Consequently it is clear from the comparison between these results that little can be deduced concerning the quantities of plasma protein taken up by tissues when the protein is labeled by administering to the donor animals a single labeled amino acid like serine-β-C^{14}. The results obtained at any given time interval will depend on the rate of uptake of the plasma protein by the tissue, its rate of breakdown to free amino acid and on the *rate of catabolism of the particular labeled carbon atom (s) concerned.*

Summary. It is possible to produce generally labeled plasma protein with very high specific activity in rats by feeding them suitable bacteria *(Rhodospirillum rubrum)* cultured in media containing labeled bicarbonate. These bacteria have protein in which all the amino acids present are labeled. The metabolism, in the recipient rats, of the C^{14} in the plasma protein from these donor animals (B) has been investigated and compared with that already described(1,2,7) for plasma protein labeled by feeding donor rats serine-β-C^{14} (A), with the following results: (1) The disappearance of the C^{14}-labeled protein B from the circulation, following its intravenous injection, follows the same curve as that found when protein A was employed. (2) The appearance of $C^{14}O_2$ in the respiratory gases is significantly less in these animals following the parenteral injection, but more following the oral administration than in recipients A. (3) As compared with A, the tissues of recipients B contain more C^{14}-labeled protein no matter which route of administration is employed, and this is particularly true when the C^{14}-labeled protein is given orally.

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Concentration of a Hyperglycemic Factor from Urine of Schizophrenics. (19288)

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The presence of a hyperglycemic factor in the urine of so-called schizophrenic patients has been reported by Meduna and Vaichulis (1). Meduna and McCulloch(2,3) have reclassified these schizophrenics as "oneiro-

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phrenics" and have claimed that a correlation exists between the presence of a hyperglycemic factor in the urine and an abnormal carbohydrate metabolism as demonstrated by a resistance to insulin, a pseudo-diabetic reaction to the Exton-Rose test, and a protracted sugar-tolerance curve during an intravenous glucose-tolerance test.

This study was undertaken to isolate, and possibly characterize, the hyperglycemic factor believed to exist in the urine of patients usually classified as schizophrenics.

Materials and methods. I. *Urine.* Urine from both male and female schizophrenic (without further classification) patients at the Western Psychiatric Institute and Clinic was collected during 4-day intervals, pooled, and preserved in the cold (0 to 3°C) with the addition of thymol. The crude starting-material was precipitated essentially according to Meduna and Vaichulis(1); by acidifying the urine with glacial acetic acid to pH 4.5 and removing the resulting scanty, flocculent sediment by continuous flow through a Sharples supercentrifuge at about 60,000 *g*. Our method for recovering this precipitate consisted in removing the contents of the rotor and homogenizing it in a glass-homogenizer (4) with sufficient water to form a slurry, which was then lyophilized(5). The resulting tan-colored powder (fraction *A*) served as the starting-material for all fractionation studies. During a 13-week period, a collection of about 425 liters yielded 47.6 g of substance *A* (average yield, 0.11 g/l). Individual urine samples varied considerably in *A* content and, in an examination of 4 individual specimens the yield of *A* varied from 0.08 to 0.67 g/l. Harrow *et al.*(6) have described the concentration of a hyperglycemic factor from acidified urine of normals by adsorption on benzoic acid. In order to ascertain whether the same type of material was involved, 25 l of pooled urine from psychotics was treated by their procedure(6), and 5.4 g of substance equivalent to Harrow's Fraction *A* was isolated; (their yield was 4.7 g per 25 l). Although the crude starting-substances obtained by the two procedures were approximately equal in hyperglycemic activity, the Meduna procedure was adhered to by reason of its simplicity and convenience in handling large volumes of urine. However, the benzoic-acid technic has been applied in a preliminary study of individual urine specimens (1 to 3 l), where it appeared to be a superior technic for clinical use.

II. *Bioassay.* The bioassay for induced hyperglycemia consisted in measuring the blood-sugar rise in fasted rats following intraperitoneal injection of the test substance. Male rats (Sprague-Dawley strain; weight 200 to 350 g) were fasted for about 16 hours prior to injection; this period seemed most satisfactory for obtaining low, consistent, fasting levels without a conversion to fat metabolism(7). The usual technic consisted in taking two 0.1-ml blood samples from a lateral tail vein opened by a transverse incision; the first sample, just prior to injection, and the second sample, 100 to 105 minutes after injection. In order partially to overcome the considerable variation in the response of the rats, a semi-quantitative estimate of hyperglycemic potency was obtained by using groups of three rats at several dosage levels. Occasionally, a rat showed little or no response. Most samples were therefore tested several times, and occasional erratic results eliminated from the calculations. It was found that, using a small dose of any of the more potent fractions, the peak of hyperglycemia occurred at 100 to 105 minutes after injection; this is in agreement with the earlier report(1) that the peak occurs at (very approximately) 2 hours after injection.

III. *Blood sugar* was measured by the Horvath and Knehr spectrophotometric modification(8) of the Folin and Malmros method (9) for the determination of blood-glucose in 0.1 ml of blood. We found that this method requires twice as much ferric ion as is recommended. Although the method measures total reducing-substance, its use was justified in that only the magnitude of change, rather than the absolute value, was desired. A semi-quantitative measure of hyperglycemic activity was arrived at by dividing the mean blood-sugar rise, ΔS, by the dosage; thus $\Delta S/mg$, (the mg % rise in blood sugar per mg of substance injected) is an index of potency. The dose-response curve was nearly linear for the

TABLE I. Fractionation and Biological Activity of a Hyperglycemic Factor from Urine of Schizophrenics.

Discard	Outline of processes	Active	Fraction	% from A	Range of doses tested mg/rat*	No. of rats	Potency ΔS/mg†	Stand. dev.
		Urine						
	Acidify to pH 4.5 and centrifuge							
Supernatant		Precipitate	*A*	100	25-85		<1	
	Alcohol and ether extractions							
Supernatant		Residue	*B*	90	30-125		1 -1.2	
	Extractions at pH 8 to 10							
Residue		Supernatant	*C*	40-45	20-60		1.8-2.7	
	Precipitate at pH 3							
Supernatant		Centrifugate	*D*	15-25	6-12	18	3.8	1.5
	Deionize at pH 8							
Resin		Effluent	*E*	10-15	5-10	20	4.8	1.7
	Adsorption with Darco G-60							
Charcoal		Filtrate	*F*	6-8	4-8	21	7.7	2.4
	Trypsin digestion and dialysis							
Dialysate		Non-dialyzable	*G*	4-5	2-4	18	14.4	5.4

* By intraper. inj. See text for studies of intrav. inj.

† Increase in blood glucose above the mean fasting level, divided by mg of material injected, reported as the range for fractions *A* through *C*, and as the means and stand. dev. for *D* through *G*.

lower doses generally used. The mean, pre-injection, blood-sugar concentration in 47 rats was 90.1 mg % with a standard deviation of 5.8. This mean was subtracted from the individual post-injection values to obtain the ΔS values, because statistical analysis showed the means and deviations to be not significantly different from those obtained by subtracting the individual fasting values from the post-injection rises.

Experimental. A schema of the procedures employed in concentrating the substance, along with the range of biological activities of the various fractions, is presented in Table I. The starting material (Fraction *A*) was not homogenous with respect to solubility in any of the common solvents tested. Of the non-aqueous solvents tried, it was found that alcohol, followed by ether extraction, removed some lipid constituents, including some of the chromogens. The first step, therefore, consisted in two extractions with absolute ethanol (100 ml/g) followed by 2 extractions with ether (50 ml/g); evaporation of the combined alcohol and ether extracts to dryness resulted in a dark-red oil which was emulsified in water with the aid of Tween 80 and, on bioassay, showed no hyperglycemic activity. The al-

cohol- and ether-extracted substance, Fraction *B*, was dispersed in water by thorough mechanical stirring and slight warming to evaporate residual ether. The dispersion was adjusted to pH 8.0 with sufficient 0.1 *N* sodium hydroxide, and centrifuged in Lusteroid tubes at 2400 *g*. The supernatant was removed, the residue redispersed in water, adjusted to pH 9, and again separated by centrifuging. After repeating the process at pH 10, the three extracts were combined (Fraction *C*). For bioassay, the resulting extract (about 50 ml per g of *A*) was neutralized to pH 7 and lyophilized. Otherwise, it was acidified directly to pH 3; this resulted in a fairly heavy, grey, flocculent precipitate which was best separated by freezing the suspension overnight and then allowing it to thaw. The cold, acid supernatant was then readily removed by centrifuging, and was neutralized and lyophilized; this acid-soluble fraction was assayed several times at levels as high as 25 mg per rat, but exhibited no hyperglycemic activity. The acid-insoluble fraction, *D*, was dispersed in water and redissolved by the addition of sufficient 0.1 *N* sodium hydroxide to keep the pH of the solution between 7.5 and 8.0. In preliminary experiments, it was found that uric acid was present in fraction *D*. Since uric acid could be separated from the hyperglycemic factor and since pure uric acid is not active at a 10-mg level, commonly employed for this fraction, a procedure was devised for its removal. The uric acid, and possibly some unidentified ionic contaminants, was removed by batchwise treatment with a mixed cationic-anionic-exchange resin (Amberlite XE-81, Analytical grade) by monobed deionization(10). The resin (1 to 2 g per g of *A*) was added in several portions to a mechanically stirred solution of *D* while the operation was observed with a pH meter. Normally, the pH would rise and then slowly fall to neutrality, at which point the suspension was filtered through a glass-wool pad. This step was repeated when the uric-acid content was high. The effluent (Fraction *E*) was still a dark-amber, somewhat colloidal solution which was biologically active. It was found that this solution could be partially decolorized with adsorbent carbon without loss of activity. Two g of Darco G-60 was added to each 100 ml of a neutral 1% solution of *E*, the suspension heated in a boiling-water bath for 6 to 8 minutes, cooled, and filtered through a Seitz bacteriological filter to remove the carbon and any suspended matter carried through to this step. The filtrate was lyophilized to a pale-grey powder, Fraction *F*.

TABLE II. Elementary Analysis of Various Fractions.

Fraction	*D*, %	*F*, %	*G*, %
Carbon	45.21	44.29	41.41
Hydrogen	6.30	5.89	6.62
Nitrogen (Dumas)	11.37	11.46	8.56
Sulfur	3.85	1.04	.31
Ash	2.93*	6.08	6.81
Cation in ash, calc. as sodium			2.49

* Probably due to fact that this sample was precipitated in an acid solution, whereas *F* and *G* were neutralized fractions.

An elementary analysis of *F* is recorded in Table II. The material gave positive biuret, ninhydrin, xanthoproteic, and Molisch tests. It was precipitated at pH 4.0 to 4.5, but was soluble below and above this region. The active substance was precipitated by sodium sulfate added to 50% saturation. Addition of zinc ions in alkaline solution caused precipitation. The ultraviolet absorption spectrum exhibited but one maximum, at 275 mμ —characteristic of proteins(11). The infrared spectrum exhibited three maxima at 3.0, 6.1, and 6.4-6.5 μ—all of which also occur predominantly in 6 known proteins examined by us and which agree with reported values (12). An ultracentrifugal analysis of substance *F* at 1% concentration in 0.1 *M* phosphate buffer, pH 7.0, gave only a single peak, the sedimentation-velocity constant of which was calculated to be 6.5 Svedberg units at 23°C. All these data are consistent with the tentative conclusion that the hyperglycemic factor is a protein or a material strongly bound to protein. In a number of studies with proteolytic enzymes it was found that *F* could be digested with *crude* trypsin (Armour 1-75), using 4% of *F* at 30-35°C for 2 hours at pH 8, without loss of activity. The digest, in a mechanically-rotated cellophane bag, was dialyzed against distilled water at room temperature for 6 hours. The non-dialyzable

solution was then heated to destroy the trypsin, cooled, filtered, and the filtrate lyophilized. The resulting pale-grey powder, Fraction *G*, which represented about 60% of *F*, was almost twice as active as *F*. The lyophilized dialysate was found inactive at a dosage of 6 mg per rat. *Crystalline* trypsin (Worthington Labs., 1 x cryst.) at a 2% level under similar conditions, produced less dialyzable material and a somewhat smaller increase in potency. The digestion mixture resulting from the action of crude trypsin on purified globulin (Armour) produced no hyperglycemia.

The elementary analysis of *G* is given in Table II. It did not show isoelectric precipitation at pH 4.0 to 4.5, nor was it precipitated by 10% aqueous trichloroacetic acid. The ultraviolet and infrared spectra were practically identical with those of Fraction *F*. The sedimentation-velocity constant was, however, significantly smaller; namely 2.7 Svedberg units at 27°C. On heating *G* in 0.1 *N* hydrochloric acid or 0.1 *N* sodium hydroxide in a boiling-water bath for 10 minutes, the hyperglycemic activity was destroyed. Studies on earlier fractions had indicated that the biological activity of a neutral solution was unaffected by heating in a boiling-water bath for 8 to 10 minutes, and that solutions adjusted to pH 3 or pH 11, prior to heating, lost less than 50% of their activity. The individual steps, as well as the complete fractionation, were repeated a number of times, with minor variations, with essentially the same results.

Results. I. Urine of psychotics. Our studies indicate that there is a substance in the urine of schizophrenics which can be concentrated 15- to 20-fold from a crude, acid-precipitated material and which produces a temporary hyperglycemia in rats when injected intraperitoneally or intravenously. The results, expressed as rise in blood sugar per mg of substance ($\Delta S/mg$), are rather variable for the crude fractions and hence are only expressed as approximate ranges in Table I. The later fractions, *D* through *G*, gave more consistent results. The potencies in a large number of rats, along with the variabilities, expressed as the standard deviations for these fractions, are presented in Table I. With the earlier fractions we noted a considerable degree of toxicity, producing limpness and diarrhea in 1 to 3 hours, and death in 10 to 24 hours in a number of animals. The higher doses of *F* and *G* often produced a transient diarrhea but rarely any other outward signs of toxicity. It was found that intravenous (tail vein) injection gave a potency about 4 times as great as did intraperitoneal injection. The peak of this hyperglycemia came at 70 to 90 minutes, so that, for these bioassays (*i.v.*), the post-injection blood samples were withdrawn after 80 minutes.

II. *Normal urine.* In a study of 21 male and female normals, Meduna and Vaichulis (1) found evidence for the excretion of a small amount of hyperglycemic substance; this gave an average rise in blood sugar showing a maximum at 1 hour after injection and diminishing thereafter. The implication was that the hyperglycemic factor may be a *normal* physiological entity which schizophrenics excrete in abnormally large amounts. Since our study was primarily concerned with the *isolation* of the substance, normal urine was of secondary interest for the present. However, as a control on our procedure, we collected two separate batches of urine from anonymous healthy males at the Mellon Institute. The urine was processed in a manner entirely analogous to that outlined for the urine of psychotics, except that the bioassays were run on fractions equivalent to Fractions *D* and *E*. The results of 3 experiments are given in Table III. As may be noted, very little, if any, hyperglycemia was obtained with the material separated from normal urine, even when tested at somewhat higher dosages than for comparable fractions of psychotics' urine, and this in spite of the fact that the yield of *D* or *E* per liter of normal urine was less than that from the urine of psychotics. Since blood sugar was measured only at 2 hours after injection, the earlier (1 hour), small hyperglycemic effect(1) was not sought for by us.

Discussion. Harrow *et al.*(6) reported on a hyperglycemic substance obtained from normal urine. The blood sugar of fed rabbits (2 kg) was raised 23% by the subcutaneous injection of 2.6 mg of his most active material.

TABLE III. Summary of Data on Urine of Normal Males.

Batch	Vol, liters	Yield			Bioassay	
		Fraction	mg/l	% from A	Dosage, mg/rat	ΔS/mg
I	41	*A*	230			
		D	14	6.1	11.5, 34	2, .9
		E	2.7	1.2	11, 17	0
II	45	*A*	250			
		E	4	1.6	18, 27	0

Our Fraction *G*, when injected intravenously in fasted rats (250 g) at a level of 0.5 and 1.0 mg/rat produced an increase in blood sugar of 35 and 69 mg %, respectively (which calculates as a ΔS of 17 for a dose of 1 mg/kg). Although the species of test animals and the conditions were different, these figures suggest that the activities of these hyperglycemic substances are probably of the same order of magnitude. However, we obtained at least 20 times as much Fraction *G* per l of urine as did Harrow *et al.* of their most active material from normal urine. Other investigators(13-15) have reported on the presence of a hyperglycemic factor in the pancreas, but, since hyperglycemia is apparently due to several physiological mechanisms, and since the mode of action of our factor is as yet undetermined, speculation on the possible relationship of these various hyperglycemic factors appears unwarranted at this time. Although the specific relationship between the presence of the hyperglycemic factor in the urine and the schizophrenic syndrome requires further investigation, our study lends support to the thesis that an abnormal biochemical pattern may be involved in schizophrenia.

Summary. The presence of a hyperglycemic factor in the urine of some schizophrenics has been confirmed. A scheme for a 15- to 20-fold concentration of the principle is described, along with data on the yield and potency of the various fractions isolated. Physical and chemical studies of the most active fractions indicate the factor to be a protein, or a material bound thereto. By a comparable fractionation of normal male urine, a measurable quantity of the hyperglycemic factor was not found.

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