specific agglutination produced a much more diffuse pattern, whereas nonspecific agglutination usually had a more definitive but rough margin and appeared in the serum controls to the same extent. Whenever possible sera were aliquoted and stored at —20°C. The antibody titers to several antigens were determined in a single test; therefore, the serum samples were not frozen and thawed more than twice prior to the determinations herein described. The sera were not kept longer than 1 day at 4°C before testing. This method of handling sera could generally prevent the occurrence of spontaneous agglutination. When spontaneous agglutination did occur, it could usually be eliminated by a second inactivation of the sera for 10 minutes at 56°C.

Summary. A new microtiter method has been developed for determination of neutralizing antibody titers for parainfluenza viruses and for typing hemadsorbing viruses. The microtiter plates are read macroscopically by hemagglutination pattern. The new method has advantages over both the conventional tube technique and a previously described micro-neutralization test. The results obtained with the new method are comparable with those of the previously described methods.

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Partial Characterization of Urine Protein with Insulin-Like Activity.* (32273)

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Previous work in this laboratory(1) confirmed the observation of Cahill, Pawan and Chalmers(2) that benzoic acid precipitates of human urine contain a factor capable of augmenting $C^{14}O_2$ production from C-1 labeled glucose by the rat epidymal fat pad. This activity was found to be increased in the urine from obese persons over that from nonobese controls and to increase during prolonged fasting(1). It was designated urinary insulin-like activity (UILA) because of the resemblance to plasma ILA.

The present paper describes a partial purification of the biological activity of an extract from urine of an obese female who excreted large amounts of UILA.

Patient material. The patient was a 27-year-old female with childhood onset of obesity. At time of examination she weighed 300 lb and was otherwise normal. Glucose tolerance was normal and no proteinuria was present. Twenty-four-hour urine collections were taken during feeding and fasting. Levels of UILA previously assayed(1) were elevated during fasting. A 24-hour sample of urine taken during fasting was submitted to the following studies.

Methods. Preparation of urine extracts. Benzoic acid precipitates of urine and the alkaline extracts were prepared for assay by the method described by Bolinger *et al*(1).

Column chromatography. An aliquot of the alkaline extract was diluted with buffer pH 7.6 (0.01 M PO_4, 0.15 M NaCl), placed in Visking dialysis bags (18/32) and dialyzed for 24 hours against the same buffer at 4°C. The bags were then transferred to 0.1 M acetate buffer at pH 4 and dialysis carried out for another 24 hours.

The samples were redialyzed against 0.05 M PO_4 buffer pH 7.6 for 24 hours, removed from the bags and lyophilized. One hundred

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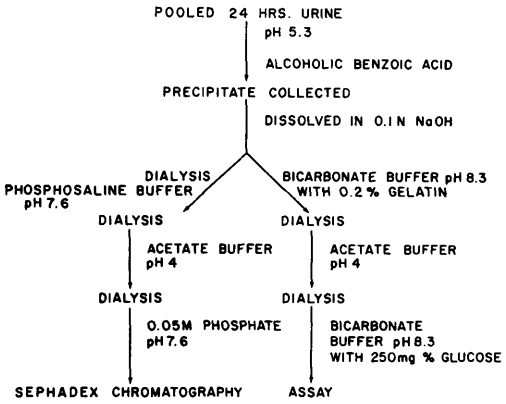

FIG. 1. Flow sheet for separation of urine insulin-like activity.

mg of the lyophilized material containing 33 mg of protein was dissolved in 5 ml of the phosphate buffer and applied to a 100 × 2.5 cm column containing Sephadex G-100 equilibrated in 0.05 M PO_4, pH 7.6.

Five ml fractions were collected at 4°C and protein concentration was indicated by absorbancy at 280 mμ. Flow rate was kept at 25-50 ml/hr. Selected fractions were dialyzed against distilled water for 18-24 hours, lyophilized and frozen.

Assay of samples. The lyophilized samples were redissolved in Gey's physiologic buffer and the insulin-like activity assayed using the experimental matrix described by Renold *et al*(3,4) for plasma ILA. The results are expressed in terms of micro units of insulin. A sample of the crude benzoic acid precipitate was dialyzed against a series of bicarbonate and acetate buffers(1) and assayed for insulin-like activity present prior to column chromatography. The processing and subdivision of the samples is outlined in Figure 1.

Neutralization studies. Incubation of the different samples with rabbit antiserum to human serum albumin or with guinea pig antiserum to bovine insulin was done in a water bath for one hour at 37°C (0.2 ml/flask).

Polyacrylamide gel electrophoresis. The method described by Ornstein and Davis(5) was used for the analysis of proteins at pH 9.5. The columns were prepared with 7.5% acrylamide for the lower gel.

Protein analysis. Protein concentrations were determined by absorbancy at 280 mμ or by a modified Folin-Ciocalteau method(6).

Results. Three protein peaks were obtained when Sephadex gel chromatography was applied to the alkaline extract of the benzoic acid precipitate (Fig. 2). Total protein recovery of the extract approximated 100% and the relative percentage of protein was as follows: Peak I, 49%; Peak II, 28%; and Peak III, 23%.

The results of polyacrylamide disc electrophoresis of the crude benzoic acid precipitate and the 3 protein peaks isolated by chromatography are shown in Fig. 3. Normal human serum is illustrated as a reference control. Several protein bands are found in the benzoic acid precipitate (B.A. ppt.), the most prominent corresponding to slowly migrating globulin and faster moving albumin. Peaks I and II displayed identical eletcrophoretic patterns and were characterized by the persistence of the heavy globulin line and virtual absence of protein in the albumin region. In contrast, Peak III appears to contain only a single protein which migrates in an identical fashion to human serum albumin.

A 3-fold concentration of specific activity

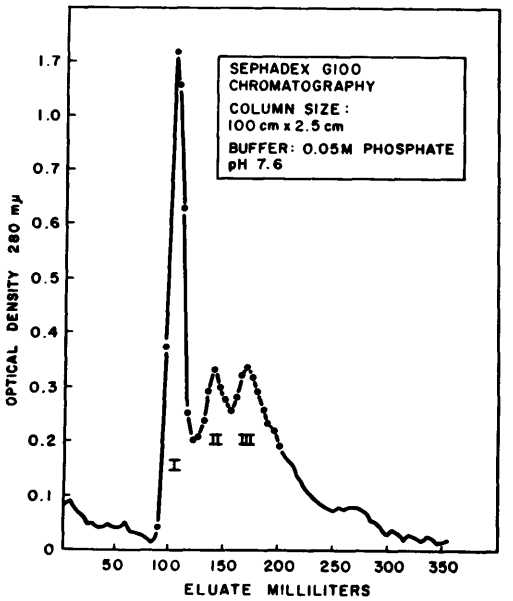

FIG. 2. Sephadex G-100 fractionation showing 3 protein peaks were obtained, referred to as Peak I, II, and III.

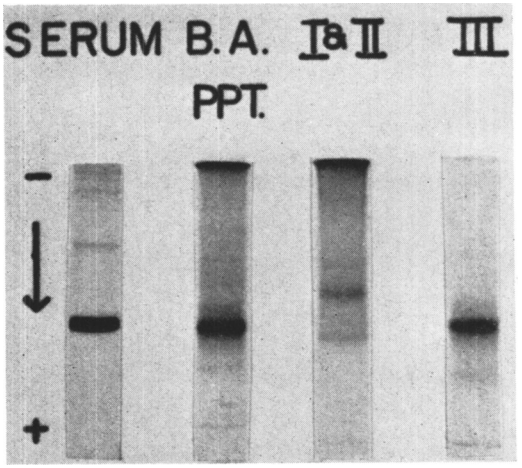

FIG. 3. Polyacrylamide disc electrophoresis Serum = normal human serum B.A. ppt. = benzoic acid precipitate Peak I, II, III.

(from 5,100 to 16,300 μU insulin/mg of protein) was obtained in Sephadex Peak III (Table I). The increased biological activity in Peak III was achieved in the presence of a decreased protein concentration.

Bovine serum albumin in concentrations equivalent to and greater than that of the protein concentration of the extract evoked only minimal biological activity in the fat pad (Table II). Thus the biologic responses from the Sephadex fractions cannot be attributed to a non-specific protein effect.

Addition of rabbit antiserum to human serum albumin (RAHSA) produced no inhibition of activity in Sephadex Peaks I and II, but produced a significant inhibition ($P<.05$) in fraction III (Table II).

Blank samples containing no protein but processed in an identical fashion to that of the urinary extracts, including the same exposure to dialysis tubing, produced neither stimulatory nor inhibitory activity in the bioassay. Incubation of guinea pig antiserum to bovine insulin with the extract and its Sephadex fractions produced no inhibition of biologic activity.

TABLE I. Insulin-Like Activity in Urine Protein.

	ILA,* μU/ml	Protein, mg/ml	Specific activity, μU/mg protein
Benzoic acid precipitate	5,100	1.000	5,100
Sephadex fractions			
Peak I	119	.216	550
" II	119	.120	991
" III	3,000	.184	16,300

* Each bioassay performed on three segments of fat pad at $P<.001$.

Discussion. The finding of insulin-like activity in the benzoic acid extracts of human urine(1) has raised questions as to the specificity of the biological assay and the nature of the material evoking the biologic response.

Rat adipose tissue is responsive to a number of factors other than insulin(7), including such non-specific ones as protein and electrolyte concentration. The present studies exclude the possibility of a non-specific protein effect as the source for most of the biological activity, since concentration of activity was obtained in the Sephadex Peak III in the face of reduced protein concentration. Furthermore, assays carried out using bovine serum albumin concentrations equivalent to, or exceeding that of the urinary extracts produced no significant biological response. Artifactual effects from the dialysis tubing(8) are excluded by blank controls.

TABLE II. Neutralization Studies.

Substance tested	Insulin-like activity, μU/ml
Bovine serum albumin, 10 mg/ml	23
" " " , 2 mg/ml	12
Fraction I	17
" I + RAHSA*	46
" II	312
" II + RAHSA	446
" III	3000
" III + RAHSA	893

All assay results valid at $P<.001$ by Analysis of Variance (Renold *et al*, 4).
* RAHSA = Rabbit antiserum to human serum albumin.

A close association of the biologic activity with albumin is suggested by the electrophoretic pattern and by the significant reduction in activity effected by antiserum to human serum albumin. A residual activity remaining after exposure to the antiserum is of the same order as that found in fractions I and II, suggesting a non-specific effect for this component.

The absence of any effect of potent guinea pig anti-insulin serum strongly militates

against insulin as a source for this biologic effect. In this respect the activity closely resembles the "atypical" serum insulin described by Samaan *et al*(9,10); however, the present characterization of UILA from an obese human subject suggests that it is more closely related to albumin than to unmodified insulin.

Summary. The insulin-like activity found in urine protein from obese subjects during fasting was characterized by benzoic acid precipitate and Sephadex column chromatography. The major portion of the urine insulin-like activity was found in a single protein peak which was homogeneous by polyacrylamide gel electrophoresis, and its electrophoretic mobility was identical to human serum albumin. This active fraction was significantly neutralized by rabbit antiserum to human serum albumin. These results suggest that the insulin-like activity in urine of obese subjects is more closely associated with albumin than with unmodified insulin.

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Effect of Aflatoxin B_1 on the Deoxyribonucleic Acid Polymerase of *Escherichia coli*. (32274)

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Aflatoxin, a mixture of 4 toxic metabolites produced by *Aspergillus flavus*, has been characterized chemically(1-3). Aflatoxin B_1, the most toxic of the aflatoxins, is a potent carcinogen in rats(4,5) and trout(6). Legator (7) and Gabliks *et al*(8) reported that aflatoxin B_1 suppressed mitosis, inhibited DNA* synthesis, and produced giant cell formations in tissue culture in a manner similar to some alkylating agents. Spectroscopy and equilibrium dialysis studies have shown that aflatoxin B_1 binds to both native and denatured DNA *in vitro* (9). Microorganisms sensitive to aflatoxin as determined by growth inhibition include strains of gram-positive, spore-forming bacilli and one streptomycete (10).

Escherichia coli contains an enzyme, DNA polymerase, deoxynucleoside triphosphate: DNA deoxynucleotidyl transferase (EC 2.7.7.7). This enzyme polymerizes deoxynucleotides, as triphosphates, into DNA and the *in vitro* synthesis requires a DNA primer (11). In a study of the effect of lysogenic induction by mitomycin C on DNA polymerase, Pricer and Weissbach(12) described a change in the priming ability of the DNA of induced *E. coli* K 12 λ cells. Other investigators have also reported that mitomycin C acts at the DNA level in *E. coli* B(13).

* The abbreviations used are: DNA, deoxyribonucleic acid; TCA, trichloroacetic acid; EDTA, ethylene diaminetetraacetic acid; TTP, thymidine triphosphate; dCTP, deoxycytidine triphosphate; dATP, deoxyadenosine triphosphate; dGTP, deoxyguanosine triphosphate.