sumably, this may reflect a critical level of adrenal cortical activity which must be attained in the rat to permit the hyperglycemic response to become apparent. In our experiment this may be attributed to an accumulation of depot cortisol over the 5-week period.

The glucocorticoid requirement for growth hormone hyperglycemia in the rat, demonstrated herewith, agrees with data indicating an adrenal steroid requirement for the inhibitory effect of anterior pituitary extract and growth hormone on glucose uptake by the isolated rat diaphragm. Growth hormone injected into adrenalectomized rats has been found incapable of inhibiting glucose uptake by the diaphragm *in vitro* unless the animals were pretreated with cortisone(12-14).

Summary. Growth hormone in a dose which regularly leads to hyperglycemia and glycosuria in normal tube-fed rats has no such effect in saline or DOCA maintained tube-fed adrenalectomized rats. Treatment with subdiabetogenic doses of cortisol restores the hyperglycemic response to growth hormone in adrenalectomized rats. These results are compared to previous studies of growth hormone effects in adrenalectomized cats and dogs.

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Acute Splenic Tumor Produced in Rats by a Mucoprotein Obtained from Rabbit Urine.* (25657)

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When mucoprotein fractions from phenylhydrazinized rabbits' urine were assayed for erythropoietic activity, it was noted that spleens of receptor rats showed marked increase in weight, within 48 hrs. after the first of 2 injections of extracts given one every 24 hours. No correlation was seen between splenic and erythropoietic response, as measured by plasma iron turnover, and uptake of Fe^{59} in erythrocytes. Greatest splenic responses were noted in rats injected with extracts of urine of normal rabbits devoid of erythropoietic activity. Histology of hypertrophic spleens was strikingly similar to that described by Rich(1,2) for acute splenic tumor, and now recognized as a characteristic response of spleen to antigens(3). In view of the magnitude of the response observed in re-

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lation to published observations and since it was a response to a soluble antigen, it was decided to study the dose response curve for this extract and its effect on plasma protein synthesis, nucleic acid content and sensitivity to irradiation.

Method. Female grey rats a x c strain (obtained from Inst. de Biologia "Juan Noe" Univ. de Chile) weights ranging from 160-180 g were used. In some experiments fasted rats were used, as this was the condition of animals in assays of erythropoietic activity; in others normal rats were employed. Rats were fasted 3 days and received intraperitoneal injection of the extracts on 2nd and 3rd day of fasting. The rats were then exsanguinated by aortic puncture on the morning of fourth day of fasting; spleens were removed and weighed to the nearest mg; then fixed in Bouin Holland. Histological sections were stained with Hematoxylin Azur II Eosin. Normal receptor rats received either one or 2 intraperitoneal injections of the extracts, one a day and were then sacrificed 48 hours after first injection. Urine was collected overnight from metabolic cages into sterile glass flasks, surrounded by ice-salt sawdust mixture. Urine was centrifuged after collection and then frozen. *Ethanol preparation of extracts.* 1 vol of normal rabbit urine is brought to pH 4.5 with 0.5 M acetic acid, then 4 volumes ethanol are added slowly with constant stirring. The urine is left overnight in the cold; the precipitate is separated by centrifugation, extracted with ether, then dried *in vacuo*. The dry precipitate is extracted with 0.01 M K_2HPO_4, and the material which does not dissolve is separated by centrifuging 20′ at 20,000 g (Lourdes AXJ centrifuge). The supernatant is used in the assays directly or after 24 hours dialysis against 100 vol, 9% NaCl in the cold. Protein content of the supernatant was determined by the Biuret method(4), and the dose injected is given in mg protein. *Amberlite treatment.* In some cases the crude alcohol extract was brought to pH 4.8 by dialysis against 0.01 M acetate buffer. To this solution 20 g Amberlite (IR-45 (OH) Analytical Reagent Fisher Sc. Co.) were added/60 ml extract and the mixture left 48 hr in the cold with constant stirring. The supernatant was then separated and tested for its effect on spleen weight and Fe^{59} distribution (5). *Kaolin adsorption.* 1 vol of urine is brought to pH 4.5 with 0.5 M acetic acid, then 20 g Kaolin (American Standard Acid Washed, Fischer & Co.) is added/liter of urine; the mixture stirred for 1 hr, left at rest for 1 hr, and the precipitate separated by centrifugation. The precipitate is then extracted with 50 ml 1 M NH_4OH/20 g kaolin, the supernatant is separated, brought to pH 4.5 with M acetic acid, then treated with 5 volumes cold acetone ($\sim -4°C$), the precipitate is washed with more acetone, then ether and dried *in vacuo* at 25°C. The dry extract is then treated as in procedure A. *Studies of C^{14} amino acid incorporation into plasma proteins.* 3 μc C^{14} uniformly labelled amino acids (obtained from hydrolysate of chlorella proteins, Radiochemical Centre Amersham, England) was injected intraperitoneally 4 hours before sacrifice of the rats. Blood was obtained by aortic puncture using isotonic citrate-citric buffer at pH 6.4 1 ml/4 ml blood, as anticoagulant. The plasma separated by centrifugation was fractionated with $(NH_4)_2SO_4$ pH 6.4, at different saturations. The fractions collected were those precipitating between 10-30%, 40-50%, and > 50%, that obtained by adding 10% T.C.A. to the supernatant of the 40-50% fraction. The ammonium sulfate precipitates were dissolved in saline, reprecipitated and washed with 10% T.C.A.; all T.C.A. precipitates were extracted with ethanol-ether, then acetone; finally the precipitates were suspended in acetone, filtered on to paper disks, weighed and counted using a thin end-window Geiger counter. A C^{14}-Polyethylene disk obtained from Oak Ridge Nat. Lab., was used as standard for checking the counting system. The results are expressed as C.P.M/mg, corrected for self-absorption using an empirical correction curve. *Irradiation.* Rats were placed in 1 mm wall aluminum, containers 5 x 5 x 15 cm, in a circle at 28.5 cm (mid-line position) from a suspended 130 curie CS^{137}. Ba^{137} γ source (obtained from Oak Ridge Nat. Labs). The exposure dose rate was measured with 100 r thimble ionization chambers, built by Lansdverk Instruments, using a model L 64 roent-

TABLE I. Spleen Weights and Erythropoietic Response of Rats Injected with Extracts of Rabbit Urine and Native Rabbit Plasma. The following abbreviations are used: Et = ethanol; Ex = extract; PH = phenylhydrazine; R = rabbit; N = normal; P = plasma; U = urine. Erythropoietic response was judged by the effects of extracts on Fe^{59} uptake by red cells(5).

Group	No. of rats	Spleen wt (mg)	Erythropoietic response
Control fasted rats	7	290 ± 10	—
Fasted rats + Et Ex PH R U	7	480 ± 20	+
Fasted rats + Amberlite treated Et Ex PH R U	7	262 ± 23.2	+
Fasted rats + Et Ex N R U	14	770 ± 4.1	—
Normal rats	7	310 ± 5.1	—
Idem + Et Ex N R U	7	918 ± 24.5	—
" + N R P	6	301 ± 14.5	—

gen meter. Total dose to which animals were exposed was 550 r, with an exposure of 73.5 minutes. Immediately after irradiation the animals received 6 mg of an ethanol extract of normal rabbit's urine, and 24 hours later another 6 mg. Groups of rats were sacrificed at 2 and 5 days after irradiation. In some irradiated animals, C^{14} AA was injected to study plasma protein radioactivity and others received Fe^{59} for study of plasma iron turnover. The 30-day mortality with 550 r was 3/24 animals in a simultaneously run experiment.

Results. Table I shows spleen weights in rats receiving 6 mg of an ethanol extract of normal and phenylhydrazinized rabbit urine. The striking increase in spleen weight in animals receiving extracts from normal rabbit urine can clearly be seen. It is interesting to note that injection of 6 ml of normal rabbit plasma in rats produces only very slight changes in spleen weight. Also shown in Table I is the result of amberlite treatment on the splenic effect of ethanol extract of urine of phenylhydrazinized rabbits; the extract maintains its erythropoietic activity, but loses its splenic effect. An example of the histological aspect of the enlarged spleens is given in Fig. 1 which shows marked hyperplasia with abundant large mononuclear cells and frequent mitosis. The D.N.A./g, 146% of controls and R.N.A./g, 160% of controls, content of the enlarged spleens is also strikingly increased, a reflection probably of the increased cellularity. Table II shows a comparison of the effects of different doses of ethanol extracts and the effects of dialyzed ethanol extracts and kaolin extracts of normal rabbit's urine. In experiments summarized here, normal rats were used as receptors and only one intraperitoneal injection of extract was given at 48 hours, after which the animals were sacrificed. It can be seen that the effect on spleen is a function of the dose of material given, that the active factor is non-dialyzable and can be obtained by adsorption on kaolin. Table III summarizes results of experiments in which C^{14} AA uptake in plasma protein fractions was studied. Ethanol extracts of rabbit's urine increase the C^{14} AA uptake in plasma proteins precipitating between 40-50% saturation of ammonium sulfate (α, β globulins). The increase in radioactivity in the 20-33% fraction (γ globulins) is slight, and specific activity of the albumin fraction is similar to that of controls. Table IV summarizes results of radiation experiments. The spleen weight response to ethanol extracts of normal rabbit urine is very markedly depressed after 550 r irradiation. There is no increase in C^{14} AA uptake in plasma protein fractions, and depression of erythropoiesis (Fe^{59} studies) is similar in con-

TABLE II. Dose Response Relation for Splenic Weight Response to Extracts of Normal Rabbit's Urine. D = dialyzed; rest of abbreviations same as in Table I. 6 rats/exp.

Group	Spleen wt
NaCl	320 ± 9.85
1 mg Et Ex N R U	560 ± 5.9
1.5	583 ± 14.6
3	672 ± 18.2
1 D Et Ex N R U	641 ± 11.9
3 kaolin ext. N R U	705 ± 19.2

TABLE III. C^{14} Amino Acid Incorporation into Different Fractions of Plasma Proteins. 6 rats per exp.

Fraction	cpm/mg
20–30% Sat. control	31.5 ± 2.20
" Et Ex N R U	41.5 ± 2.88
40–50% Sat. control	34.9 ± 2.80
" Et Ex N R U	59.2 ± 3.85
>50% Sat. control	33.4 ± 2.90
" Et Ex N R U	35.0 ± 1.12

TABLE IV. Effect of Irradiation on Acute Splenic Response to Extracts of Normal Rabbit's Urine.

			Fe^{59} (3 hr) % dose		C^{14} proteins (cpm/mg)		
		Spleen wt	Plasma	Erythrocytes	20-30	40-50	>50%
Control	2 days post irrad.	184 ± 8.3	52.1	.60	38.85	37	41.2
Et Ex N R U	*idem*	254 ± 9.8	50.9	.60	38.4	35.8	41
Control	5 days post irrad.	192 ± 5.5	43.8	.5	24.9	24.2	23.2
Et Ex N R U	*idem*	243 ± 6.9	32.7	.2	23.9	26.8	23.8
Normal rats		310 ± 5.1	17.4	4.8	31.5	34.9	33.4

trol and treated irradiated animals.

Discussion. The results presented here indicate that a solution containing a few mg of a non-dialyzable, kaolin-adsorbable, ethanol precipitable material probably mucoprotein, obtained from normal rabbit urine, can produce a dramatic increase in rat spleen weight within 48 hours after injection. The active material may be a normal component of urine or a product of bacterial contamination in the bladder; the method used to collect the urine does not allow a distinction to be made between these 2 possibilities. The histologic appearance of the spleen is similar to that described by Rich *et al.*(1,2) as acute splenic tumor, characteristic of the splenic response to foreign protein. Wissler *et al.*(3) have studied rat spleen response to particulate antigens of bacterial origin (Typhoid Friedlander) and sheep red cells. Increases in spleen weight are not as great as those described here, but microscopically similar. The greatest difference is that the injected material in our experiments is soluble, not precipitating at 20,000 g. The fact that rabbit plasma protein, in much larger doses, produces only slight response in rats indicates that the urinary material is much more powerful antigenically than plasma constituents.

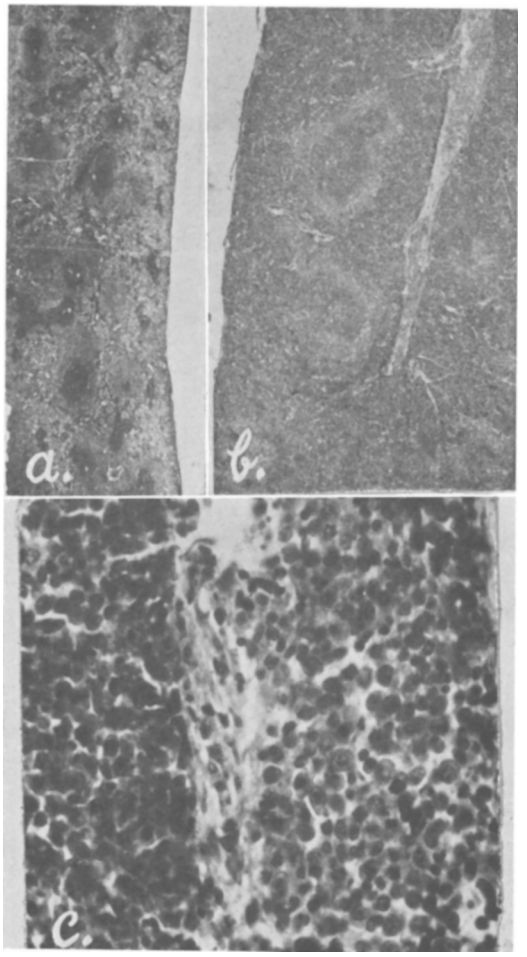

FIG. 1. Histologic aspect of spleen in *(a)* normal rats (× 15), *(b)* spleen of rat inj. with ethanol extract of normal rabbit's urine (× 15) and *(c)* spleen of same rat *(b)* (× 400). Note homogeneous aspect of spleen of treated rats, its great cellularity and presence of many large mononuclear cells.

The finding of an increase in the C^{14} amino acid uptake especially in the plasma proteins precipitating at between 40-50% saturation, (α, β globulin) is surprising in that one would have expected rather to find an increase in γ globulin fraction, which only showed a slight rise. The fact that specific activity of albumin in both control and treated rats was similar, would indicate probably that increased uptake of C^{14} AA in other fractions is a reflection of increased synthesis, rather than of a change in the amino acid pool.

The marked depression of the splenic and disappearance of the plasma protein response caused by 550 r irradiation accords with the finding of others as to the radiosensitivity of

the splenic system reacting against antigens (6).

Further purification and chemical characterization of the material responsible for the acute splenic response observed, will be of utility in clearing up the mechanism of the cellular response to purified antigens of one chemical species.

Summary. Extracts of normal rabbit urine obtained by ethanol precipitation or kaolin adsorption produce marked increase in rat spleen weight. Histologic examination of spleens shows marked hyperplasia with abundant mononuclear cells of the type described as acute splenic tumor cells. The splenic response is a function of dose of protein given. Animals treated with these extracts show significant increase in C^{14}-amino acid uptake, specially in the α, β globulin fraction. Preirradiation of the rats (550 r Cs γ) inhibits splenic and plasma protein response to extracts of normal rabbit's urine.

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Effect of BAL (2,3-Dimercaptopropanol) on Hypoglycemic Action of Insulin. (25658)

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Patterson(1) described the diabetogenic action of dehydroascorbic acid (DHAA). Simultaneously, Patterson(2) and Dominguez (3) found that by injecting compounds containing sulfhydryl groups, the animals were protected against the diabetogenic action of DHAA. Intravenous administration of DHAA produces a characteristic response which consists of 2 consecutive periods. The first, a violent convulsive period, starts immediately after intravenous injection of DHAA and is prolonged for no more than 3 minutes. The animals immediately enter the second period, a depressive stage of 5-10 minutes duration. The convulsive period coincides with a deep hypoglycemia. When BAL was injected intramuscularly, the rats were protected against the hypoglycemic response as well as against development of the experimental diabetes induced by dehydroascorbic acid(3). The initial hypoglycemia is probably due to a massive release of insulin from the islets of Langerhans, similar to the release of insulin which has been suggested to follow alloxan injections (4). Since BAL inhibited the initial hypoglycemia produced by DHAA, the effect of BAL on the hypoglycemia produced by exogenous insulin was studied.

Methods. Rabbits of similar weight were divided into 4 groups and fasted for 12 hours before experiment. Blood sugar was determined by Nelson's method(5) and values obtained were considered as normal control levels. Doses of insulin and BAL used in the experiment were 0.7 I.U. and 70 mg/kg of body weight, respectively. The different groups were treated as follows: *Group a* received intravenous injection of insulin. *Group b* received intramuscular injection of BAL. *Group c* were injected intramuscularly with BAL. Thirty minutes later the animals received intravenous injections of insulin. *Group d* received intravenous insulin and intramuscular BAL simultaneously. Blood glucose was

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