cant ($p = <.01$). These observations suggest that mice inoculated with pertussis vaccine become sensitive to the lethal effects of histamine by virtue of the reduced ability of their tissues to inactivate this amine.

H. pertussis vaccine may have a toxic effect on the enzyme, presumably histaminase, which destroys histamine. This vaccine may also exert its effect indirectly by damaging the adrenal gland. The tissues of an adrenalectomized animal have a reduced histaminase activity(7). In their sensitivity to histamine, to bacterial vaccines and to anaphylaxis, pertussis inoculated mice are very similar to adrenalectomized animals(2).

Summary. Lung tissue from pertussis-inoculated, histamine-sensitive mice has a reduced ability to destroy histamine as compared with lung tissue from uninoculated, histamine-resistant animals.

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Influence of Hypophysectomy and Growth Hormone on Cartilage Sulfate Metabolism.* (20726)

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The biochemical effects of growth hormone on cartilage are virtually unknown, yet the proliferative response of epiphyseal cartilage to growth hormone is one of the most impressive biological affects of this endocrine substance when administered to immature hypophysectomized rats. The recent demonstrations(1,2) of the uptake of radioactive sulfate by cartilage and its incorporation into chondroitin sulfate suggested that this process might be profitably explored as to its endocrine control. Toward this end, the effects of hypophysectomy and growth hormone injection on the uptake of radioactive sulfate have been examined relative to the normal animal and are here described.

Methods. A tracer dose of S^{35} labeled inorganic sulfate containing 1.5×10^7 counts per minute in a 0.1 ml of saline was injected into the saphenous vein of ether anaesthetized rats. Blood samples were removed after 13, 19 and 25 hours of equilibration and the heparinized plasma was separated by centrifugation. Plasma samples were deproteinized with an equal volume of 10% trichloracetic acid and the inorganic sulfate precipitated with an ethanolic solution of benzidine(3). The twice ethanol-washed precipitate of benzidine sulfate was then determined colorimetrically with β-napthoquinone sulfonate(4). An aliquot of the dissolved benzidine sulfate solution was plated on a copper planchet and radioactivity estimated at virtual infinite thinness. For the determination of the specific activity of cartilage sulfate (inorganic and esterified), samples of rib cartilage were dissected free of adherent tissues and 40-50 mg were digested with 3 ml 4 N HCl for 2 hours in a boiling water bath after which the samples were evaporated to dryness. Solution of the residue in 5 ml of water was almost complete and any undissolved carbonized residue was removed by centrifugation and discarded. The pH of the solution was between 2.0-2.5. The sulfate was precipitated with ethanolic benzidine, filtered on paper and air-dried. The radioactivity of the benzidine sulfate precipi-

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TABLE I. The Effect of Growth Hormone on S^{35} Specific Activity of Plasma and Cartilage.

Rat type*	Hr after S^{35} inj.	Plasma S^{35} specific activity†	Cartilage S^{35} specific activity	Cartilage specific activity‡ / Plasma specific activity
Normal	13	2.8	2.6	.93 (.6 –1.3)
Hyp		14.1	.5	.04 (.03– .04)
Hyp + GH		11.4	1.1	.10 (.08– .11)
Normal	19	1.5	2.5	1.7 (1.4 –1.8)
Hyp		9.9	.7	.07 (.04– .09)
Hyp + GH		6.5	2.3	.35 (.3 – .4)
Normal	25	1.4	2.0	1.4 (1.0 –1.8)
Hyp		4.0	.6	.15 (.1 – .2)
Hyp + GH		3.9	1.8	.46 (.4 – .6)

* 4-5 rats in each group.
† Specific activity = counts/min. × 10^4/mg sulfur.
‡ Avg value and range of variation.

tate on the filter paper was measured and suitable corrections for self-absorption applied since mass of the precipitate was appreciable. All samples were corrected for decay. The benzidine sulfate precipitate was estimated colorimetrically as in the case of plasma.

Rats of the Long-Evans strain were hypophysectomized at 26 days of age and injections with either growth hormone or saline began on day 12-13 post-operative. For comparison with untreated hypophysectomized rats, normal control rats 28 days of age were used. All animals were females and weighed 70 g with the maximum variation being ±5%. All animals were maintained on Diet 1(5) fed *ad libitum* during the experiment.

The growth hormone was prepared essentially by the Li salt fractionation method(6). The minimum effective dose for thyrotropic and interstitial-cell stimulating activities in the growth hormone preparations used was 4 mg as determined by the 4-day histological repair test using immature hypophysectomized rats. The minimum effective dose of growth hormone necessary to evoke a significant epiphyseal cartilage response in the 4-day tibia test (7) was a total dose of 10 μg. Hypophysectomized rats treated with growth hormone were given 25 μg daily for 3 days following which labeled sulfate was injected one hour after the last growth hormone injection.

Results and discussion. In Table I are summarized the plasma inorganic sulfate and cartilage inorganic and esterified sulfate specific activities after 13, 19 and 25 hours.

Hypophysectomy of the immature growing rat markedly decreases the uptake of S^{35} by costal cartilage. When the cartilage:plasma sulfate ratios of hypophysectomized and normal rats are compared, it can be seen that in the hypophysectomized rat S^{35} uptake is never more than 11% of normal. The greatly diminished uptake of the tracer by hypophysectomized rat cartilage presumably is responsible in part for the relatively smaller dilution of the injected tracer.

As it is evident from cartilage:plasma specific activity ratios shown in Table I, growth hormone has a small but nonetheless unmistakable effect in restoring the ratios toward normal. Thus, at 19 and 25 hours after injection of the tracer, the specific activity ratios in the growth hormone treated rats were 21% and 33% of normal respectively. Uptake in the untreated hypophysectomized rats was 4% and 11% at the corresponding time intervals.

The restoration of the specific activity ratio in the growth hormone hypophysectomized rat has not been attained under the present experimental conditions. It is possible that a longer period of treatment with a higher dose level of growth hormone may be required. Furthermore, the well known synergism between growth hormone and thyroxin may necessitate the combined injection of the two hormones for the complete repair of cartilage sulfate metabolism in hypophysectomized rats.

Summary. Hypophysectomy of immature growing rats results in a markedly diminished sulfate uptake of costal cartilage as judged from the cartilage to plasma inorganic sulfate specific activity ratios. The injection of

growth hormone partially restores this ratio to normal under the conditions employed. The plasma inorganic sulfate specific activities of hypophysectomized rats are several fold higher than those of normal rats.

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A Fibrinolytic System in Human Milk.*† (20727)

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The presence of an activator of plasminogen in urine suggested a search for fibrinolytic activity in other fluids secreted or excreted through narrow channels(1). Milk and casein are known to contain small amounts of a proteolytic enzyme(2). The experiments to be presented here show that there exists in human milk a fibrinolytic system resembling that demonstrated in human blood(3). This result is in accordance with the independent observation by Geiger(4), that streptokinase increases the proteolytic activity of mixtures of milk with rabbit plasma.

Materials and methods. Enzymic activity was estimated by the following 3 methods: I. Fibrinolytic effect was estimated on bovine fibrin containing plasminogen by the standard fibrin plate method(5) and recorded as the product (in mm^2) of 2 diameters of the lysed zones (average of 3 determinations). II. Effect on bovine fibrin, where plasminogen had been destroyed by heating, was estimated similarly on fibrin plates heated for 45 min. at 85°C(6). III. Proteolytic activity was estimated on casein (3% in borate or phosphate buffer) after precipitation with perchloric acid (final concentration 6%) by determination of the optical density of the supernatant at 270 mμ using a Hilger Uvispek spectrophotometer.

Human milk was obtained fresh from The Children's Hospital, Fuglebakken, Copenhagen. The samples were centrifuged, and the skimmed milk was used. Bovine plasminogen was prepared from ammonium sulfate precipitated fibrinogen(5), using 0.9% NaCl instead of the usual barbital buffer) by heating for 10 minutes at 54°C followed by dialysis of the supernatant against saturated ammonium sulfate. The precipitate was redissolved, dialysed against borate buffer, and lyophilized. Streptokinase ("Varidase") was obtained from the Lederle Laboratories. Buffers: Barbital: 0.1 M; pH 7.75; NaCl to $\mu = 0.15$. Borate: 0.05 M; pH 7.75; NaCl to $\mu = 0.15$. Phosphate: 0.06 M; pH 7.35; $\mu = 0.15$.

Fibrinolytic activity of human milk. A wide range of fibrinolytic activity was encountered when samples of human milk were applied to standard fibrin plates. Activity measurements of about 400 mm^2 were obtained maximally and a considerable number of samples showed no or only a slight activity. In all samples of milk the addition of streptokinase produced a high fibrinolytic activity. The streptokinase solution alone had only a very slight effect on the standard fibrin plates (Fig. 1). However, no or only a trace of activity was found when the effect

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