# Effect of Insulin on Subcutaneous Absorption of Drugs in Rats.* (25572)

GAETAN JASMIN,† AND ANDRE BONIN
*Dépt. d'Anatomie Pathologique, Université de Montréal, Montreal, Canada*

Previous studies have shown that insulin, in minimal doses, increases rat's sensitivity to dextran edema(1). It was assumed that insulin activates the reaction by other means than hypoglycemia, possibly by enhancing penetration of dextran into the tissues. This led us to investigate the effect of insulin on urethane anesthesia and on convulsions produced by dimethyl-p-phenylenediamine(2). The influence of insulin on subcutaneous absorption was also assessed by intravenous administration of Evans blue in animals already injected intradermally with physiologic saline.

*Materials and methods.* Seventy-two female rats (Holtzman) weighing 140 to 160 g were fasted 6 hours before absorption tests. In each experiment, animals were divided into groups of 12 and received 0.2 I.U. of zinc-insulin (Connaught), subcutaneously, 15 minutes before injection of other substances. Ethyl urethane or dimethyl-p-phenylenediamine was given subcutaneously at 150 mg and 4 mg, respectively, each in 0.2 ml of saline. Degree of anesthesia and convulsions was appraised visually according to arbitrary scale of 0 to 4+. Readings were made at 10, 20 and 30 minutes after injection of drugs and our results are expressed as percentages of maximal possible response (4+). For salt solution absorption test, 0.2 ml of physiologic saline was injected intradermally into 3 different regions of dorsal skin. This was followed by intravenous injection of 0.2 ml of 0.5% solution of Evans blue. Ten minutes later, animals were sacrificed and dorsal skin was dissected from underlying muscle and turned over for appraisal of intensity of blueing at site of injection of physiologic saline (Fig. 1). Readings were made according to relative scale of 0 to 4+. Since blueing was inversely proportional to rate of absorption, our percentual values (Table I) were calculated as follows: 0 = 100; + = 75; 2+ = 50; 3+ = 25; 4+ = 0. A few minutes before animals were killed, 1 ml of blood was taken from jugular vein for sugar determinations made according to method of Folin Wu.

*Results.* Mean values (Table I) reveal that activity of urethane was definitely increased by insulin. The promoting effect on anesthesia was readily demonstrable during 30 minutes following injection of relatively small doses of urethane. After this period, the differences were less evident, apart from slow recovery of insulin-treated rats. In dimethyl-p-phenylenediamine, the effect of insulin was more clear cut: latent period of convulsions was reduced by 10 minutes. Although blood sugar values were significantly lowered by insulin treatment, they remained within the lower limits of normality.

Our observations from intradermal saline tests are in line with above findings. The percentage of absorption, as judged by low degree of blueing reaction, was markedly increased in insulin-treated rats (Fig. 1).

*Discussion.* These results support the concept that insulin enhances absorption rate of substances injected subcutaneously. Thus, it seems reasonable to assume that the promot-

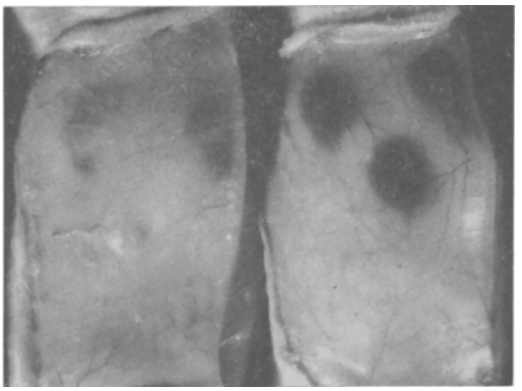

FIG. 1. Effect of insulin on subcut. absorption of physiologic saline. Relative blueing reaction at sites of subcut. inj. of saline. *Left*, insulin-treated rat. *Right*, control.

* This work supported by grants from Canadian Arthritis and Rheumatism Scc. and Fn. Joseph Rhéaume of Montreal, Canada.

† Medical Research Associate of Nat. Research Council of Canada.

TABLE I. Effect of Insulin on Induction of Urethane Anesthesia, Dimethyl-p-Phenylenediamine Convulsions and Subcutaneous Absorption of Physiologic Saline in Rats.

| Groups of 12 rats | Urethane* anesthesia (%) 10 min. | 20 min. | 30 min. | Glycemia,† mg % | PPD‡ convulsions (%) 10 min. | 20 min. | 30 min. | Glycemia,† mg % |
|---|---|---|---|---|---|---|---|---|
| Controls | 0 | 13 | 25 | 112 ± 3.1 | 0 | 0 | 90 | 128 ± 4.2 |
| Zinc-insulin (0.2 I.U. s.c.) | 25 | 37 | 67 | 78 ± 5.9 | 0 | 90 | 100 | 80 ± 5.3 |

| | Absorption rate of saline (%)§ | Glycemia,† mg % |
|---|---|---|
| Controls | 39 | 118 ± 3.7 |
| Zinc-insulin (0.2 I.U. s.c.) | 80 | 74 ± 6.1 |

* Mean readings following s.c. inj. of 100 mg/100 g of ethyl urethane.
† Mean ± S.E.
‡ Mean readings following s.c. inj. of 2.6 mg/100 g of dimethyl-p-phenylenediamine.
§ Mean percentage based on intensity of blueing 10 min. after i.v. inj. of 0.2 ml of 0.5% Evans blue.

ing effect of insulin on dextran reaction is also derived from increase in absorption. Whether insulin influences the reaction by changing permeability of cells or of ground substance remains to be shown. It has been reported that subcutaneous absorption of urethane is augmented by cortisone, in rats, and by dehydration and fasting, in mice. This was interpreted as resulting from a reduction of hyaluronic acid content in connective tissue ground substance(3). Insulin may act similarly, perhaps by influencing activity of hyaluronidase.

Fasting, especially in adrenalectomized rats, augments sensitivity to dextran(1), and similarly, activity of various drugs was more uniform in animals subjected to a short period of fasting. It is also common knowledge that the efficiency of therapeutic agents, even when given parenterally, is higher in fasting patients. That a slight decrease in glycemia, within physiologic range, activates absorption phenomena may also explain the fact that insulin given at a low dosage promotes absorption of substances injected subcutaneously.

*Summary.* Subcutaneous absorption of urethane and of dimethyl-p-phenylenediamine was augmented by insulin, as evidenced by acceleration of anesthesia and convulsions caused by these substances, respectively. Insulin also promoted absorption rate of intradermally injected physiologic saline.

1. Jasmin, G., Bois, P., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 656.
2. ———, *Proc. Can. Physiol. Soc.,* Meet. Toronto, June 9, 1959.
3. Hvidberg, E., Schou, J., *Nature,* 1959, v184, 646.

Received December 7, 1959. P.S.E.B.M., 1960, v103.

## Potassium Metabolism in Rats with Chronic Renal Insufficiency.* (25573)

ASHTON B. MORRISON (Introduced by J. Russell Elkinton)
*Dept. of Pathology, School of Medicine, University of Pennsylvania, Philadelphia*

Male rats which had been subtotally (5/6) nephrectomized developed a form of chronic renal insufficiency similar in many ways to that occurring in the human patient with chronic nephritis. At death, lesions were found in the cardiovascular system and in bones of the rats, which were indistinguishable in quality from those found in some patients with chronic renal insufficiency(1). When at least one month had elapsed following operation the adrenals were enlarged(1). This enlargement in such animals suggested that during period of renal insufficiency the adrenals

* This work was supported by U.S.P.H.S. grants from Nat. Inst. of Arthritis and Metab. Dis., Bethesda, Md., and from Smith, Kline, and French Fn.