exceeded 14.55 ppm, there was a pronounced increase in bone F content. In fetal bones, the increase appeared at lower levels of supply, *i.e.* 5.2-14.55 ppm. In the blood, F levels were similar in pregnant and non-pregnant rats. At intakes exceeding 14.55 ppm, there was only a slight increase in blood F in both groups. In the urine, F levels increased in both groups similarly, and rather steeply with increased F intake, thus indicating immediate removal of any F surplus with the urine in pregnant and non-pregnant rats. It is stressed that this metabolism is different from that observed in pregnant women, who tend to retain an F surplus during pregnancy.

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Effects of Insulin on Adrenaline and Noradrenaline Content of Some Rat Tissues.* (26934)

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It is well known that injection of insulin can deplete the suprarenals of adrenaline(1, 2,3). In contrast to these results, administration of insulin has been found to increase considerably the adrenaline content of the heart and liver(3).

Recently we have found that the adrenaline content of several organs is markedly increased in rats after injection of large doses of adrenaline(4). It therefore seemed possible that the increased amounts of adrenaline found in the heart and liver following insulin administration were due to accumulation of adrenaline released from the suprarenal glands. To test this hypothesis, the effects of insulin on adrenaline and noradrenaline contents of hearts and salivary glands were studied in animals from which the suprarenal medullae had been removed. Demedullation does not itself alter the catecholamine content of the organs studied(4).

Methods. Male albino rats of the Sprague-Dawley strain weighing 300 to 400 g were

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TABLE I. Effects of Insulin on Adrenaline* and Noradrenaline* Contents of Hearts and Salivary Glands under Various Experimental Conditions.

Procedures	Hr of insulin	Hearts		Submaxillary glands		Parotid glands	
		Adren.	Noradren.	Adren.	Noradren.	Adren.	Noradren.
Suprarenals intact	0	.05 ±.004	.55 ±.040	.11 ±.009	1.23 ±.073	.06 ±.006	1.04 ±.086
		n = 11		n = 13		n = 12	
	3	.16 (.13-.23)	.69 (.61-.83)	.24 (.20-.30)	1.47 (1.08-1.90)	.20 (.12-.36)	1.14 (1.09-1.23)
		n = 3		n = 3		n = 3	
Suprarenals demedullated 1 wk	0	.06 ±.007	.62 ±.033	.11 ±.005	1.41 ±.057	.08 ±.009	1.05 ±.069
		n = 10		n = 16		n = 16	
	3	.04 (.03-.05)	.57 (.43-.80)	.05 (.04-.07)	1.55 (1.35-1.83)	.04	1.03
		n = 3		n = 4		n = 1	
	5	.01 (.00-.02)	.61 (.53-.73)	.02 (.00-.03)	1.53 (1.45-1.62)	.00 (-.02-.01)	1.21 (1.04-1.46)
		n = 3		n = 3		n = 3	
	7	.02 (.00-.04)	.19 (.07-.28)	.02 ±.006	1.61 ±.117	.01 ±.009	1.02 ±.070
		n = 4		n = 11		n = 8	
	11	.01 (.00-.02)	.15 (.04-.27)	.02 (.00-.05)	1.42 (1.05-1.69)	.01 (-.02-.03)	1.09 (.81-1.20)
		n = 4		n = 4		n = 4	
Ecolid, 10 mg	7	.05 (.04-.05)	.55 (.42-.63)	.09 (.08-.10)	1.44 (1.21-1.60)	.06 (.04-.10)	1.13 (.87-1.35)
		n = 3		n = 3		n = 3	

* μg/g of tissue (wet wt). Mean ± S.E. or range (in parentheses). n = No. of extracts assayed.

used throughout. Bilateral adrenal demedullation was performed one week prior to administration of insulin and estimation of tissue catecholamines. Estimations of catecholamines were made according to the spectrofluorometric method described by Bertler, Carlsson and Rosengren(5). Extracts were prepared from the pooled tissues of 2 animals so that each extract contained material from 4 salivary glands or 2 hearts. For further technical details see Strömblad and Nickerson(4).

Insulin (zinc-insulin Toronto 80 I.U./ml) was given subcutaneously in a dose of 10 I.U. per 100 g body weight. No food was given, but glucose (3 ml of a 5% solution) was injected intraperitoneally when coma or convulsions developed.

Results. In the experiments of Hökfelt (3), the largest increase in adrenaline contents of the heart and liver was found 3 hours after injection of insulin. To confirm this observation under our experimental conditions, a group of rats with intact suprarenal medullae was examined 3 hours after injection of insulin. In these animals the adrenaline contents of the heart and the salivary glands were considerably increased (Table I).

In contrast, adrenaline levels of heart and salivary glands of adrenal-demedullated rats were found to be decreased at various time intervals following injection of insulin (Table I). The noradrenaline content of the salivary glands was unaltered at all of the time intervals studied, and that of the heart was decreased only 7 and 11 hours after insulin.

Injection of chlorisondamine (Ecolid) (10 mg in 1 ml 0.9% NaCl solution intraperitoneally) one-half hour before administration of insulin into adrenal-demedullated animals prevented the changes in catecholamine content otherwise found 7 hours after injection of insulin (Table I).

Discussion. The data presented above confirm the observations of Hökfelt(3) that administration of insulin to intact rats can

cause an increase in the adrenaline contents of various tissues. However, the adrenaline levels were decreased consistently by insulin in animals from which the suprarenal medullae previously had been removed. These results provide strong support for the hypothesis that the increased amounts found in intact animals represent catecholamine released from the suprarenal medullae in response to the insulin-induced hypoglycemia and subsequently accumulated from the blood stream in the tissues studied.

The mechanism of action of insulin in reducing the adrenaline content of organs of suprarenal-demedullated rats is not entirely clear. However, the observation that ganglionic blockade prevents the effects of insulin suggests that these are mediated *via* the autonomic nervous system. This does not imply that all tissue storage sites for adrenaline are directly under the control of the autonomic nervous system. It is possible that a part of the effect of insulin on the adrenaline contents of tissues of suprarenal-demedullated animals is due to a flooding of the organism with noradrenaline which might displace some of the stored adrenaline. We previously have shown that injection of noradrenaline can decrease the adrenaline content of the organs studied(4).

The results presented above demonstrate that a catecholamine released from one site can be transported to and accumulated in other organs and tissues. This observation requires that events in other areas of the body, particularly in the adrenal medullae, as well as local synthesis, storage and release, be considered in interpreting the results of tissue analyses for adrenaline and noradrenaline.

Summary. Injection of insulin into intact rats increased the adrenaline contents of hearts and salivary glands. However, it decreased the adrenaline content of these tissues in animals from which the suprarenal medullae had been removed. The noradrenaline content of the heart was decreased after 7 to 11 hours of insulin action, but that of the salivary glands was unaltered. It is concluded that the increased amounts of adrenaline found in tissues of intact animals are due to the release of this catecholamine from the suprarenal medullae and its subsequent transport to and accumulation in the organs studied.

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Immunohistochemical Identification of Tissue Culture Cells. (26935)

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A major problem in tissue culture is the identification of the cells being cultured. This is important both in connection with the various cell lines now in existence and also in analysis of new cell lines derived from fresh tissue. In the case of existing cell lines there have been accidents in which cell lines have been contaminated by other propagated cultures(1,2,3,4), and passed on to other laboratories. These contaminations have been identified by chromosome morphology, immunological technics, and viral infectivity studies.

In the case of cells growing from a primary culture, where most of the cells appear to be fibroblasts, it is important to determine whether cells unique for the tissue in question are also in culture. We have used cultured muscle cells as a model system and have