treatment with DHT, epilation greatly aggravates the otherwise inconstant and mild cutaneous calcinosis of the scalp; it can even induce lesions in an area not otherwise affected, such as the back. In both these regions, the skin is transformed into hard, heavily calcified plaques. On histologic sections (fixed in alcohol-formol and stained with von Kóssa's method), calcium deposition is seen mainly in the subepithelial connective tissue fibers and the basement membrane region (Fig. 1). Of course, on von Kóssa stained sections, it is impossible to state with certainty whether the mineral deposits are within or around the structural elements just mentioned. Yet, in following the early stages of tissue calcification, the impression is gained that the collagen fibers swell while they become gradually impregnated with calcium salts. On sections stained with Weigert's elastica stain, it is evident that simultaneously with this calcification in the collagen, the elastic fibers become fragmented. All these changes are reminiscent of human scleroderma, especially the so-called Thibierge-Weissenbach type, which is characterized by calcification.

During the 10 days following their appearance, these plaques are gradually demarcated from the underlying dermis and cast off. Then, cicatrization and sclerosis ensue. After the damaged part is shed, the skin underneath becomes re-epithelialized but remains partly or completely hairless during the following 3 weeks of observation.

Summary. In rats, a cutaneous calcinosis with sclerosis, not unlike that seen in certain types of clinical scleroderma, can be produced at will in predetermined regions of the skin. This is best accomplished if, at a critical time of systemic dihydrotachysterol overdosage, the selected cutaneous area is lightly traumatized by epilation.

1. Selye, H., *J.A.M.A.*, 1932, v99, 108.
2. ———, *J. Invest. Dermat.*, 1957, v29, 9.
3. Leriche, R., Jung, A., De Bakey, M. E., *Surgery*, 1937, v1, 6.
4. Leriche, R., Jung, A., Sureyya, C., *Presse méd.*, 1935, v43, 777.
5. Leriche, R., Jung, A., *ibid.*, 1935, v43, 1361.
6. Selye, H., *Z. Urol.*, 1957, v50, 440.
7. Selye, H., *Virchows Arch. f. path. Anat.*, 1932, v286, 91.

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Biological Activity of Blood Insulin Complexes Examined by Rat Diaphragm Tissue Assay.* (25856)

KARE GUNDERSEN† AND HARRY N. ANTONIADES (Introduced by D. M. Surgenor)
Boston Dispensary and Dept. of Preventive Medicine, Tufts School of Medicine, Boston, and Protein Fn. Labs., Jamaica Plain, Mass.

Antoniades *et al.*(1-3) suggested that insulin in blood and pancreas is largely bound by other proteins and, more specifically, by basic proteins. The insulin-basic protein complexes could be adsorbed on cationic exchange resin, in the sodium form, and could be extracted from the resin by elution with acid or alkali(3). The present communication describes studies on biological activity of insulin-complexes extracted from human blood with use of cationic exchange resin (Na^+ form). Two types of preparations have been examined for insulin-like activity by the rat diaphragm tissue assay as described by Vallance-Owen and Hurlock(4). The data indicated that the biological effect of insulin in its complex form examined *in vitro* by the diaphragm assay differs significantly from the biological effect of insulin in the "free" form, liberated from the complex at pH 9.8. Preliminary data suggest that, in contrast to the rat diaphragm assay, adipose tissue assay can

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TABLE I. Bioassay of Resin Eluates by Rat Diaphragm Tissue Assay.*

	Before pH change		After pH change	
Lot No.	Glucose uptake in μg/10 mg dry diaphragm ± SE	Insulin-like activity in mu/l of plasma	Glucose uptake in μg/10 mg dry diaphragm ± SE	Insulin-like activity in mu/l of plasma
016	42 ± 18	1.35	184 ± 25	32.9
	−82 ± 10	.0	204 ± 29	82.6
	−96 ± 5	.0		
	58 ± 29	.486		
	2†± 4	.0	116 ± 8	14.0§
	4†± 5	.0	106 ± 7	20.2§
	10 ± 10	.0		
			264 ± 11	83.4
			152 ± 12	35.1
		(48.6)‡		
		(40.5)‡		
052	6 ± 11	.0	278 ± 13	51
		(20)‡		

* All determinations were made in triplicate and calculated as net glucose uptake above control (buffer alone).

† Dialyzed against Gey and Gey's bicarbonate buffer(6) overnight.

‡ Activity in parentheses obtained on same sample by rat adipose tissue assay. Assays for insulin-like activity were kindly carried out by Drs. A. E. Renold and J. Steinke.

§ Low recoveries may result from incomplete dissociation of the complex or high pH values. pH adjustment was made without use of continuous recording pH meter.

determine insulin in its complex form.

Materials and methods. Resin eluate, lot 016 has been prepared by acid elution (0.1 N H_2SO_4, pH 2.0) from cationic exchange resin (Na^+ form) following blood collection(2). Resin eluate, lot 052 has been prepared by elution with 0.1 N NH_4OH, pH 10.5(3). The pH of the eluates was adjusted to 7.2 followed by dialysis against 0.02 M NaCl and lyophilization. *Rat diaphragm tissue assay*: based on increment in glucose uptake by this tissue in presence of insulin(4). *Rat adipose tissue assay*: based on effect of insulin in production of $C^{14}O_2$ from glucose-C^{14} by this tissue(5). *Dissociation of insulin from its complex* in eluate concentrates was carried out at pH 9.8. Lyophilized resin eluates containing the insulin complexes were dissolved in Krebs' bicarbonate buffer or in Gey and Gey's bicarbonate buffer(6). pH of the solution was brought to 9.8 under continuous stirring with use of 0.5 N NaOH, which was added slowly and dropwise. A continuous recording pH meter (Radiometer, Copenhagen, Denmark) was employed in these studies, and particular care was exercised not to exceed pH 10. The mixture was centrifuged at 4°C for 30 minutes at 3000 rpm, and pH of the supernatant fluid was adjusted to 7.2. The supernatant fluid was dialyzed overnight against the buffer employed in the assay and was examined for insulin-like activity. Control experiments of the untreated eluate, dialyzed against the same buffer were carried out simultaneously.

Results. Table I shows the results. The insulin-like activity in its complex form does not exhibit full biological activity on the rat diaphragm tissue assay. Insulin-like activity was demonstrated upon liberation of insulin from its complex by centrifuging at pH 9.8.

Biological assays for insulin-like activity of identical untreated preparations carried out by the rat adipose tissue assay(5) showed that this assay could determine insulin-like activity in its complex form (Table I). Although the reasons for the differences between the 2 assays are still obscure, one may speculate that adipose tissue can utilize insulin in its complex form whereas such complexes are not utilized by rat diaphragm tissue.

These observations have been made in an isolated and highly purified system. It is possible that in blood serum, as in the resin eluates, different fractions of insulin-like activity are determined by the different assays employed for estimation of the insulin-like activity.

Summary. Insulin complexes prepared from human blood showed no significant in-

sulin activity when such preparations were examined by the rat diaphragm tissue assay. After dissociation of insulin from its complex(es) at pH 9.8 insulin-like activity could be demonstrated by this assay. Identical preparations examined by the rat adipose tissue assay showed that this tissue could manifest insulin-like activity in the presence of the insulin complex itself. A difference is thus evident in the mechanism of utilization of the insulin complex(es) by these 2 different tissues.

1. Antoniades, H. N., Beigelman, P. M., Pennell, R. B., Thorn, G. W., Oncley, J. L., *Metabolism: Clin. and Exp.*, 1958, v7, 266.

2. Antoniades, H. N., *Science*, 1958, v127, 593.

3. Antoniades, H. N., Renold, A. E., Dagenais, Y. M., Steinke, J., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 677.

4. Vallance-Owen, J., Hurlock, B., *Lancet*, 1954, v1, 68.

5. Martin, D. B., Dagenais, Y. M., Renold, A. E., *Lancet*, 1958, v2, 76.

6. Gey, G. O., Gey, M. K., *Amer. J. of Cancer*, 1936, v27, 45.

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Transplantability Changes of Mouse Mammary Tumors After Passage Through Tolerant Homologous Recipients.* (25857)

CARLOS MARTINEZ, HARVEY KELMAN AND ROBERT A. GOOD
Depts. of Physiology and Pediatrics, University of Minnesota Medical School, Minneapolis

Transplantation of normal and neoplastic tissues in highly inbred strains of mice is ruled by classical principles of inheritance. Tumors taken from animals of one inbred strain can be successfully transplanted into the same individual (autologous graft) or into members of the same strain (isologous grafts). In addition, by crossing mice of 2 different strains, the resulting F_1 hybrids accept tumor transplants taken from individuals of either parent strain. Finally, F_2 hybrids or backcross hybrids to the non-susceptible parent accept the tumor with a frequency which will depend on number of histocompatibility factors required by the particular tumor employed (See review by Little)(1). Barrett and Deringer first reported that after passing a tumor through F_1 hybrids produced by outcrossing the strain of origin times another resistant strain, transplantability of this tumor usually increased when tested in F_2 or backcross hybrids between the same 2 strains used to produce the intermediate F_1 cross(2). The change was demonstrated to be permanent even when the tumor was returned to the original strain(3) and quite specific for the particular genotype employed in the F_1 passage (4). This phenomenon first explained by its discoverers as due to "adaptation" of the tumor, was later thought to be due to "immunoselection" of certain simplified antigenic types of cell populations preexisting in the tumor(5). However, E. and G. Klein(6,7) could not confirm cell selection interpretation. Experiments using preimmunized animals led these investigators to favor the hypothesis that the change in transplantability induced by passage through the F_1 hybrid might be the result of a change in response of the tumor to specific isoantibodies it provokes in the F_1 individual. They also found that the Barrett and Deringer phenomenon could be induced in tumor cells enclosed in cell impermeable diffusion chambers placed into the peritoneal cavity of F_1 hybrid individuals. From these experiments they concluded that the F_1 hybrid effect is probably humoral in nature(8). Using the phenomenon of acquired immunological tolerance we have studied transplantability changes of tumors after passing these tumors through homologous mice previously made tolerant of the strain of animals from which the tumors derived, and compared these results with those

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