the existence of relationships which seem to have been largely neglected of recent years, and which must be more fully investigated before we can feel that the problem of the inheritance of various human characteristics has been adequately treated.

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## Reactions of Subcutaneous Tissue to Sodium Ricinoleate and Other Foreign Substances.

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The purpose of this study was to ascertain whether there is a specific cytological response of subcutaneous tissue to detoxified toxin as compared with the reaction to a pure toxin. For further comparison of the reactions, one of the least toxic colloidal dyes was used.

Adult rabbits were injected subcutaneously with single doses of 1 cc. of 1% trypan blau in distilled water, 1 cc. of 1% sodium ricinoleate in distilled water, 1 cc. of diphtheria toxin in 1/500 dilution, and 1 cc. of diphtheria vaccine, *i. e.*, a combination of sodium ricinoleate and diphtheria toxin as prepared by Dr. W. P. Larson of the University of Minnesota. Subcutaneous tissue spreads were made by the spread method used by Maximow, von Möllendorf and others, and were fixed immediately in Zenker-formol. The stains used were Dominici's Eosin-Orange G and Toluidine Blue, Maximow's Hematoxylin Azure II-Eosin, Heidenhain's Iron Hematoxylin, and Weigert's Iron Hematoxylin. Tissue was taken at 45 minute, 1, 2, 3, and 4 day intervals for direct comparison of the effect of each of the named substances.

Very little difference in the appearance of the site of injection was noted in the 45 minute stage in the majority of cases. There were a few cases in which the area of injection turned greyish white when soap, toxin or vaccine was used, and then gradually became mildly inflamed. Some of the animals chewed at the irritated spot and produced an open sore, but no abscesses were found. When incision was made 45 minutes after injection, the subcutaneous tissue was very soft and gelatinous with a considerable quantity of fluid which probably was due in part to the undiffused irritant. The tissue was of pearly white color except with trypan blau, which had stained everything intensely blue.

In a few animals, 1 day after injection of each of the irritating substances, the area of injection became firm and thickened, and could not be raised from the muscular layers as could be done with the others. In such cases, the tissue was taken as near the site of injection as possible. The subcutaneous tissue was abundant, fairly gelatinous, and pearly white after injection of the soap, toxin, and vaccine, and blue after injection of trypan blau.

The 2 day stage was very similar to the one day stage with all the irritants used except with trypan blau, when the tissue was lighter blue.

The 3 and 4 day stages were very much alike in gross appearance, being somewhat thickened but not as firm as in the one day stage. The tissue in the trypan blau material was only faintly blue.

On microscopic examination the subcutaneous tissue from the trypan blau injected animals at the 45 minute stage showed, when it was dehydrated, cleared and mounted without further staining, that the cellular and fibrous elements had been diffusely colored by the vital dye as has been described by Downey. At this early stage the stained preparations did not show signs of inflammatory activity except for the presence of the dye particles in the cytoplasm of some of the fibroblasts and clasmatocytes. No polymorphonuclear leucocytes were seen.

In the corresponding stage in the soap material the general appearance was very similar, except that apparently empty vacuoles were present in the cells instead of dye particles.

Spreads made of toxin and vaccine injected subcutaneous tissues had more lymphocytes in some cases than did either the soap or trypan blau material, and the majority of these had very pycnotic nuclei. The cytoplasm of some of the larger lymphoid type of cells was very vacuolated, but contained no visible inclusions. There were definite signs of necrosis in the toxin injected tissue spreads.

With each of the irritants, polymorphonuclear leucocytes were most abundant one day after injection. The lymphocytes and polyblasts were fairly numerous in some spreads, with some variation between the individual animals.

On the second day there were great numbers of polyblasts, comparatively few typical fibroblasts and clasmatocytes, and fewer polymorphonuclear leucocytes than in the previous stage. The latter were breaking down and being phagocytozed by the polyblasts, clasmatocytes and fibroblasts. The soap, toxin and vaccine materials as well as the trypan blau injected subcutaneous tissue showed very little difference in reaction, a difference which did not sur-

pass the variation in reaction of the individual animals to the same irritant.

On the third and fourth days the polyblasts were still the predominating cell type. There were but few typical clasmatocytes and fibroblasts present. The only marked difference with the substances used was the visibility of the ingested dye in the vacuoles of the cells in the trypan blau material, and the apparently empty vacuoles in the cells of the soap, vaccine and toxin materials.

These results would lead one to the conclusion that the soap and diphtheria-soap vaccine are not much more irritating to the subcutaneous tissue of rabbits than is trypan blau, which is considered the least toxic of the vital dyes. The diphtheria toxin alone, although no more specific in its effect on the cellular response, produces more necrosis than the other irritants used.