bodies detectable by PCA (titres, 1/50–1/200) and by passive haemagglutination (titres, 1/160–1/640).

These results demonstrate that a hapten conjugate of poly-D-lysine is non-antigenic in strain 2 guinea pigs, whereas a homologous conjugate of poly-L-lysine is antigenic. In experiments to be reported elsewhere⁸, it was found that a saline extract of strain 2 guinea pig spleens was incapable of degrading a lightly coupled fluorescein-PDL conjugate. These findings may be considered to support the view⁹ that macromolecular antigens must first be degraded *in vivo* in order to induce an immune response. However, it is possible that even sufficiently low molecular weight hapten-PDL peptides may still not be antigenic because of their inability to undergo a subsequent specific metabolic step which is necessary for the induction of the immune response.

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Hepatotropic Effect in Mice by an Aqueous Extract of Ultra-violet-irradiated Linolenic Acid

Toxic materials are obtained by oxidation of highly unsaturated fatty acids, and significant effects are produced by these products on biological systems. Methyl linoleate and methyl linolenate are readily oxidized on exposure to ultra-violet radiation to yield products which inhibit enzymatic reactions¹ and cellular respiration² and interrupt division of yeast cells³. Other consequences of the autoxidation of lipids are detailed in a recent symposium⁴. This communication deals with an *in vivo* response of the mouse liver to an aqueous extract of ultra-violet-irradiated linolenic acid.

5 g of linolenic acid (99 per cent purity, Hormel Foundation, Austin, Minn.) were irradiated with ultra-violet light for 18 h, extracted with water and processed according to the procedure described previously⁵. Washing with organic solvents, however, was omitted. The extract gave a characteristic pink coloration with the thiobarbituric acid (TBA) reagent⁶. The pink coloration increased linearly with the duration of the period of irradiation of lipid up to 18 h⁷. Therefore, in the present work linolenic acid, after irradiation for 18 h, was extracted with water. The extract after concentration by lyophilization gave a TBA value of approximately 50 absorbance units per ml. at 532 mμ. Such an extract was injected intraperitoneally into mice at an approximately LD₂₅ level. Similarly, an extract of non-irradiated linolenic acid was also prepared.

Thirty-seven one-month-old C₅₇B₆ female mice, fed Purina Laboratory Chow and water *ad libitum*, were given two injections of 0.3 ml. of the extract of irradiated linolenic acid at 4 h apart. Two control groups (37 mice each) received similarly two injections of 0.3 ml. of the extract of non-irradiated lipid or distilled water.

The group of mice injected with extracts of irradiated lipid showed a transient loss in weight of approximately 10 per cent during the first week. No losses in weight were observed in the other groups; by the end of two weeks, all mice were of comparable weight. At least

four animals from each group were killed at various intervals by breaking their necks.

No gross changes were noted in the appearance of the animals, although 10 out of 37 mice died during the first 96 h after the injection of the extract of irradiated lipid. Eight days after the injection of the extract of irradiated lipid, the livers appeared slightly swollen. After 20 days, the two major lobes were distinctly swollen and the margins of the lobes were thick and rounded. Both these lobes had fused in mice killed within 30 days after injection and by 50 days the median, right, and left lateral lobes were fused. These animals showed loss of hair in a small area of the skin (1 cm² approximately) near the site of injection. The fused liver lobes in three mice showed adhesions to the spleen and right kidney, but the peritoneum was unaffected. The weight of the liver in percentage of the body-weight of all the mice killed after the injection of the extract varied from 6.4 to 7.5, but such variations were also observed with the animals killed from the control groups.

Microscopically, these livers showed irregular size of hepatic cells with crowding of trabeculae and enlarging of the individual lobules. The nuclei varied greatly in size, shape and staining characteristics. The cytoplasm of most of the cells was coarsely granular. Many of the liver cells had double nuclei in either mitotic or amitotic divisions; distortion of the structure of the liver and compression of the sinusoids were evident. The alteration of liver cells was accompanied by a greater prominence of Kupffer cells. In general, these livers exhibited hypertrophy and hyperplasia of both cell types.

The sections of fused livers showed the two lobes were separated by a septum. The disarranged trabecular pattern representing each of the fused lobes was entirely different. The septum appeared thin and discontinuous in fused livers of mice killed from 50 days and onwards after the injection of the extract. In some instances the interrupted areas in the septum were occupied by the hepatic cells.

Other organs (spleen, kidney, heart and brain) of mice in this experimental group failed to show distinct pathological changes. Similarly, livers from mice injected with either the extract of non-irradiated linolenic acid or distilled water exhibited no such changes.

It appears, therefore, that the ultra-violet-irradiated linolenic acid contains water-soluble materials which possess the property to induce hyperplastic changes in the liver and destructive changes in the connective tissue of the liver capsule. The exact nature of the materials responsible for these effects is not known; however, the presence of carbonyl compounds and peroxides has been ascertained.

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