EFFECT OF THE PROLONGED ADMINISTRATION OF 1:2:5:6-DIBENZANTHRACENE ON THE RETICULO-ENDOTHELIAL SYSTEM OF RABBITS

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The present communication is concerned with the effect of prolonged administration of a carcinogenic compound, 1:2:5:6-dibenzanthracene, upon the functional level of the reticulo-endothelial system (R. E. S.) of rabbits. Virgin rabbits, 20 in number, from a stock employed in previous experiments received injections of a 0.3 per cent solution of 1:2:5:6-dibenzanthracene in olive oil or benzene at intervals of ten to twenty days for a period extending, in some instances, over more than twelve months. The dosage for each animal was 1.0 c.c. or 2.0 c.c., the total dosage thus being in the order of 70 mg. of the hydrocarbon. Young rabbits are extremely sensitive to the injections and with larger quantities or shorter intervals deaths are frequent.

The functional level of the reticulo-endothelial system was estimated by the Congo red method (Stern and Willheim's modification of Adler and Reimann's test, 5, 6), and expressed in terms of the Congo red index, i.e., the rate of disappearance from the circulation of a known quantity of the dye, given intravenously. A high index indicates rapid absorption of the dye. Determinations were carried out before the first injection of hydrocarbon and ten days after it, and were subsequently repeated at intervals of fourteen to twenty-eight days.

RESULTS

General Effects: Most animals withstood the administration of the solution for long periods, though some died after the first or second injection and adverse effects were noticed in the survivors. Necrotic processes occurred at the site of the injections in several rabbits, but at the present writing (December 1938) have not been followed by the development of neoplasms. The general condition of the animals declined and cachexia developed but proved to be reversible when (in 8 instances) the injections were interrupted for periods of about sixteen weeks. Neither of the solvents, when injected alone, produced any adverse effect, with a single exception: in one animal receiving benzene alone a necrotic area developed at the injection site.

The Congo Red Index: Except in one animal neither olive oil nor benzene alone produced any significant changes in the activity of the R. E. S. even when the administration was carried out for a period of six months. In one animal the index rose gradually following the administration of benzene, but since a similar increase may occur in untreated animals the rôle of the benzene is questionable. 1:2:5:6-Dibenzanthracene, on the other hand, tends to lower the Congo red index, as is shown in Table I and in Figs. 1 and 2, representative

1 From The Laboratory, 37, Great Cumberland Place, London.
2 This number does not include some animals that died very soon after the first injection.
of observations in 4 animals. In one animal no fall of the index was noted. This rabbit remained in normal health for the three months during which it received injections of the compound dissolved in benzene. Resumption of injections after a period of interruption in two animals (e.g., Rabbit 14, Fig. 2) did not cause a renewed fall.

While, with the exceptions noted, the general trend of the index curves was similar in all experimental animals, the fall in R. E. S. activity differed considerably in extent. Quantitative comparisons are difficult, however, because of initial differences, the "base line" of reticulo-endothelial activity showing much individual variation. A further source of variation is related to the conditions of the experiment. With benzene as a solvent, the fall in the index was preceded by a distinct rise; with the oil solution, on the other hand, no preliminary rise was observed.

When the index began to fall, a condition of lipaemia invariably developed; this was not determined quantitatively, but inspection of the blood samples after centrifugation and before determination of the Congo red concentration revealed the high fat-content.

The prolonged administration of the compound produced changes in certain endocrine glands. Ovarian changes were seen in 5 animals, 2 of which were killed seven and nine months respectively after the first injection, while the other 3 died at various stages of the experiment. In these animals one or both ovaries contained one to three haemorrhagic follicles such as are often seen after the injection of human pregnancy urine or its extracts. Histologically, however, the ovaries did not show the advanced development usually associated with the production of haemorrhagic follicles by gonadotropic hormones; no normal corpora lutea or luteinized zones were seen in the follicles. Indeed, in three cases, degenerative changes were noted.

The thyroid was affected in 3 of 5 animals examined, showing atrophy of the follicles with or without interstitial fibrosis. Changes in the other endocrine glands were too variable or too slight to be worth recording.

The spleen was examined histologically in 5 animals. Except for areas of hyaline degeneration in one instance, no gross changes were noted.
Figs. 1 and 2. Effect of 1:2:5:6-dibenzanthracene dissolved in benzene (upper figure) and in oil (lower figure) on the Congo red index of rabbits.

In Fig. 1 (above) the solid line represents Rabbit No. 3; the broken line Rabbit No. 7. In Fig. 2 (below) the solid line represents Rabbit No. 17; the broken line Rabbit No. 14. The arrows indicate interruption and subsequent resumption of injections. The initial point in each instance indicates the base-line of R. E. S. activity, i.e., the congo-red index before treatment with 1:2:5:6-dibenzanthracene was begun.
The experiments recorded show that 1:2:5:6-dibenzanthracene tends to depress the functional level of the R. E. S., and that this depression occurs before the appearance of any malignant growth. In this respect they indirectly corroborate statements of other authors who have found degenerative changes in the R. E. S. in the presence of transplantable tumors or neoplasms produced by carcinogenic agents (1-4). It is possible that precancerous changes developed in the experimental rabbits concomitantly with the fall of the Congo red index, but escaped detection, although the animals that were killed or died were carefully dissected and most of the organs were examined histologically.

No causal relationship between the damage to the R. E. S. and the carcinogenic properties of 1:2:5:6-dibenzanthracene is yet proved, and it may be that the effects observed in the present experiments can be reproduced by the use of related but non-carcinogenic compounds, two of which are now being tested.

As pointed out above, the response to injection differed according to the solvent used. While both benzene and oil solutions ultimately lowered the index (except in one rabbit), an initial stimulating action was observed with the benzene solution that was absent when oil was employed. It is unlikely that this difference is due directly to the solvents, since neither of them produced any comparable change in the activity of the R. E. S. It is more probable that the difference is to be explained by the rate of absorption, oily deposits being absorbed more gradually than deposits of benzene.

The appearance of lipaemia cannot yet be profitably discussed. It should be noted, however, that a close relationship between fat and cholesterol metabolism and the R. E. S. has been said to exist by some authors (10, 11); also that relative lipaemia, with reduction in the concentration of lipolytic ferment, may occur in association with the development of malignant conditions (12, 13, 14).

It has been shown previously that the activity of the R. E. S. can be either raised or lowered according as certain extracts of the anterior lobe of the pituitary are administered (7, 8, 9). A comparison of the indices obtained in experiments with pituitary extracts and with 1:2:5:6-dibenzanthracene shows that the carcinogenic compound does not lower the index with the same rapidity as negatively restropic pituitary extracts. In some rabbits prolonged administration of the carcinogenic compound was required to produce indices as low as those observed after one or two doses of strongly negative restropic extract.

In view of the results obtained with restropic extracts of pituitary origin the question arises whether the effect of the carcinogenic compound establishes itself via the pituitary, i.e., by inhibiting secretion of positive restropin. Some of the observations under discussion make it seem possible that pituitary function was affected in the experimental animals; both the changes in the ovaries and the regressive changes in the thyroid suggest interference with normal pituitary function. The changes in the ovaries in particular cannot easily be regarded as of purely local origin.
Summary

Repeated determination of the functional level of the R. E. S. by means of the Congo red method shows that 1:2:5:6-dibenzanthracene tends to reduce the activity of the R. E. S. of rabbits. Lipaemia develops concomitantly with the fall in the Congo red index, and degenerative changes may occur in the ovaries and the thyroid.

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References


The writers learn from a personal communication from Dr. K. Stern and Dr. J. Gerstel that these investigators have carried out experiments with benzpyrene similar to those described here, with corresponding results.