The similarity in the immunoelectrophoretic precipitin arcs and the demonstration of the presence of antibody activity for thyroglobulin is presumptive evidence that the γ-C proteins in human serum, identified by monkey antisera, is the same class of proteins identified as γ-C by the horse antiserum Blue Boy.

Goodman *et al* noted that in the thyroglobulin system, the sensitivity of the method combining immunoelectrophoresis and radioautography was less than that originally reported with insulin(5). In the present study the level of sensitivity was not a limiting factor for the qualitative findings reported because of the use of high titered thyroiditis sera.

In an attempt to improve the specificity of the methods, the iodinated thyroglobulin extract was precipitated in an excess of a monkey antiserum to human serum proteins (T795). This antiserum forms precipitin arcs with proteins of α and β mobility as well as with the immunoglobulins β_2A, β_2M and γ-globulin of normal human serum(2). Thus any contribution of radioactivity to the precipitin arc of the radioautograph by nonspecific binding of isotope labeled serum proteins in the thyroid extract would be minimized. This procedure diminished the faint radioactivity in the precipitin arcs of the normal sera to almost imperceptible arcs without notably diminishing the *very strong* radioactivity in the precipitin arcs obtained with the thyroiditis sera.

Conclusions. The human γ-globulins, γ-A and γ-B, identified by monkey antisera, and γ-C, identified by both monkey and horse antisera, have antibody activity. In 3 sera from patients with chronic thyroiditis, the γ-A, γ-B and γ-C were shown to have antithyroglobulin activity by radioautography with I^{125} labeled thyroglobulin after immunoelectrophoresis.

ADDENDUM: After submission of this paper, an abstract has appeared which confirms the observations made here (Fed. Proc. 23:454, 1964—W. D. Terry and J. L. Fahey).

Dr. Sheldon Dray provided monkey and horse antisera and Dr. Howard C. Goodman supplied thyroglobulin extract and thyroiditis sera.

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Immunological Sympathectomy and CCl_4 Hepatotoxicity.* (29305)

ROBERT E. LARSON, GABRIEL L. PLAA AND MICHAEL J. BRODY
Department of Pharmacology, College of Medicine, State University of Iowa, Iowa City

A causative role has been ascribed to the sympathetic nervous system in production of the hepatic lesion seen after CCl_4 intoxication(1-3). One of the principal observations that led to this suggestion was that in rats, spinal cord transection at the level of C-6 or C-7 prevented the appearance of the centrilobular necrosis after an oral dose of CCl_4 (1-5). We have since shown that an alternative explanation could be advanced for the protection afforded by cord-transection, *viz.* reduced hepatic metabolism resulting from the hypothermia induced by the cordotomy (5). Our observations cast considerable doubt on the idea that sympathetic discharge alone

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is responsible for the development of hepatic necrosis seen after CCl_4.

The development of a method for immunological sympathectomy of rats(6) provided a new experimental tool for examining the possible relationship of the sympathetic nervous system to the hepatotoxicity of CCl_4. If our alternative explanation was the correct one, *normothermic* rats devoid of a sympathetic nervous system should not be protected from the effect of CCl_4.

Methods. Newborn male Sprague-Dawley rats (Simonsen Laboratories) were sympathectomized immunologically by injection of an antisympathetic nerve growth factor (ANGF)† according to the method of Brody (7,8). When these animals had reached the age of 3 months (about 300 g) several were randomly selected for adrenal demedullation. Litter-mate controls which had not received ANGF were concomitantly selected for adrenal demedullation. The demedullated rats were employed 2 weeks after surgery. At time of experiment the rats were separated into 4 groups of 4 rats each. The groups were as follows: 1) rats which had received only ANGF, 2) rats which received ANGF *and* had been adrenal demedullated, 3) rats which had been adrenal demedullated only, and 4) rats which had neither received ANGF nor been demedullated. One rat from each group was selected for testing of sympathetic function according to the method described by Brody(7). This involved measurement of hind-quarter perfusion pressure responses to electrical stimulation of the sympathetic nerves. The remaining rats in each group received CCl_4, 1.25 ml/kg, p.o., in an equal volume of corn oil. Twenty-four hours later the animals were killed and samples of liver taken. These were fixed in cold, neutral-buffered formalin for histological examination by light microscopy, after hematoxylin and eosin staining; duplicate slides were stained with Oil-Red-O in order to demonstrate fat.‡ The thoracic and lumbar sympathetic chains and the adrenals were also taken for examination by light microscopy.

Results. Fig. 1 is illustrative of the results observed. CCl_4 produced distinct centrilobular necrosis in the livers of the rats which had been treated with ANGF only (Fig. 1A). The hepatic necrosis in these animals was accompanied by less hydropic degeneration than was usually seen in normal rats after CCl_4. In normal rats the necrosis was usually circumscribed by a 1- or 2-cell-deep layer of swollen, vacuolated cells.

After CCl_4, centrilobular necrosis was also seen in the livers of rats which had undergone adrenal demedullation, whether or not the animals had previously been treated with ANGF (Fig. 1B,C). Fewer cells seemed to be involved in the frank coagulative-type necrosis. However, adrenal demedullation tended to shift the pattern of damage to one of more hydropic degeneration. The net result was that more total tissue appeared to be involved in degenerative change and progression to a more severe lesion seemed imminent. To illustrate the differences between these treatments and cordotomy, Fig. 1D is a section taken from a C-7 transected rat(4) 24 hours after the same dose of CCl_4. This animal had become hypothermic and was protected. We have never observed necrosis after CCl_4 in rats which had been C-7 cordotomized and which were hypothermic (rectal temperature about 25°C)(4,5). None of the treatments (ANGF, ANGF plus demedullation, demedullation only) resulted in hepatic lesions by itself. The animals after all of the treatments were normothermic. Oral administration of CCl_4 as described did not alter body temperature as measured by rectal probe thermistor.

The liver sections stained for fat with Oil-Red-O revealed considerable amounts of fat in all cases where CCl_4 had been administered. No apparent differences in distribution or amount were evident among the groups. In general, the fat was localized as fine to large droplets in the peripheral one-third of the lobule. The cells that had undergone hydropic degeneration contained correspondingly less fat, as did the cells in the necrotic areas.

† Antiserum was graciously supplied by Dr. R. K. Richards, Abbott Laboratories, North Chicago, Ill.

‡ Histological examination was performed by Dr. Louis M. Crews, Hazelton Laboratories, Inc., Falls Church, Va.

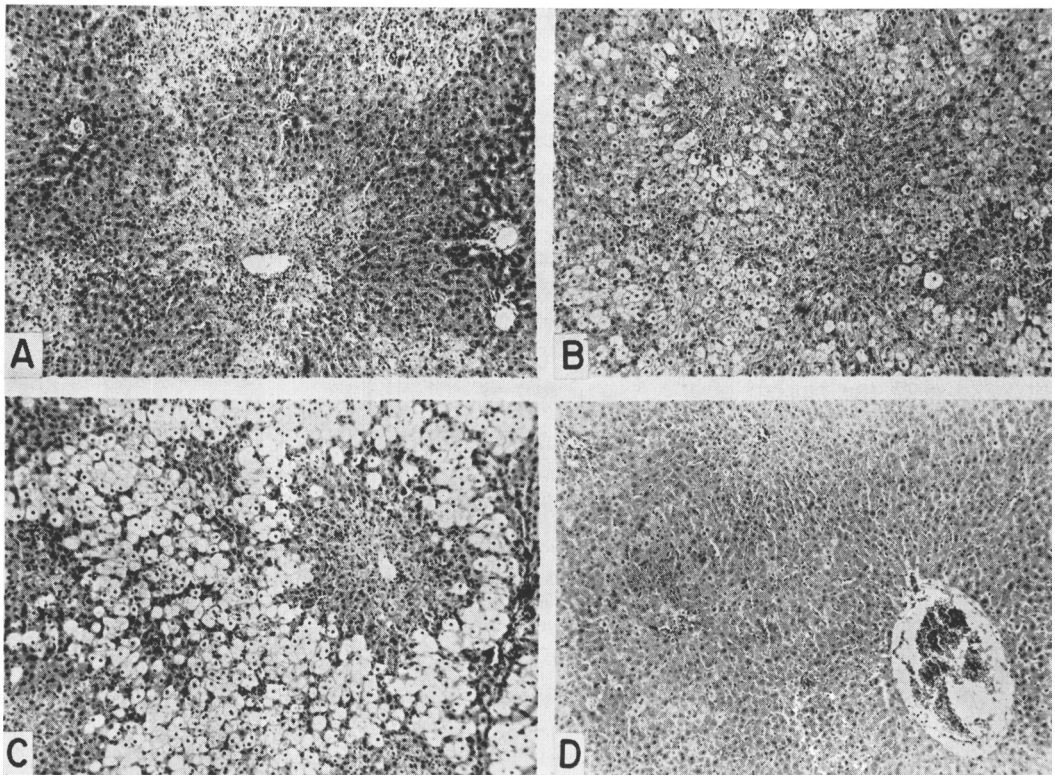

FIG. 1. Liver sections taken from rats 24 hours after CCl_4, 1.25 ml/kg, p.o. (A) Centrilobular necrosis with minimal hydropic degeneration in rats which had only been pretreated with ANGF. (B) Centrilobular necrosis and extensive hydropic degeneration in rats which had received ANGF and had been adrenal demedullated. (C) Centrilobular necrosis and extensive hydropic degeneration in rats which had been adrenal demedullated only. (D) The typical protection seen in C-7 spinal transected rats.

Those animals treated with ANGF were considered to be functionally devoid of a peripheral sympathetic nervous system because litter mates treated with ANGF did not exhibit spontaneous electrical activity along the lumbar sympathetic chains(8) and because electrical stimulation of the chains did not result in vasoconstriction of the hind-quarter vessels. Histological examination of the sympathetic chains revealed essentially complete destruction of the ganglion cells in those rats which had received ANGF. The antiserum was specific for the ganglia and did not affect the adrenal medulla. That demedullation was complete in the appropriate rats was also verified in the histological examination.

Discussion. The results presented here indicate that a functional peripheral sympathetic nervous system is not essential to the development of the typical hepatic response to CCl_4 intoxication. Centrilobular necrosis, hydropic degeneration and extensive fatty change were evident after CCl_4 in all animals whether or not they possessed functional sympathetic nerves and/or adrenal medullas. These results, in conjunction with our earlier observation that the hepatotoxicity of CCl_4 can be produced in cervically cordotomized rats under certain experimental conditions(5), substantiate the idea that central sympathetic discharge in and of itself is not responsible for the development of the lesion.

However, it appears that the presence or absence of the adrenal medulla alone may modify the hepatic response to an oral dose of CCl_4. Normally the necrotic areas are circumscribed by only a 1- or 2-cell-deep layer of hydropic cells. In demedullated rats the hydropic degenerative zone is much more extensively developed, suggesting that hydropic

degeneration and necrosis may proceed along different pathways. The role played by the adrenal medulla in the development of this degenerative response is not defined. Nonetheless, the rather striking shifts in the pattern of response merit further study.

Summary. Immunological sympathectomy does not protect rats against the hepatotoxic properties of CCl_4. This substantiates the idea that sympathetic discharge in and of itself is not responsible for development of the lesion. However, adrenal demedullation seems to alter the pattern of the lesion, in that hydropic degeneration becomes more extensive after demedullation.

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Cytochemistry of Inclusion Bodies in Tissue Culture Cells Infected with Rabies Virus.* (29306)

ROBERT LOVE, MARIO V. FERNANDES AND HILARY KOPROWSKI

Department of Pathology, Jefferson Medical College, and The Wistar Institute, Philadelphia, Pa.

Contradictory reports have been published regarding the nature of the inclusion bodies in cells infected with rabies virus. Wolman and Behar(1) found Feulgen-positive material in some inclusion bodies in the hippocampal cells of mice infected with street virus and Moulton(2) made similar observations regarding the Negri bodies in the brain of rabid skunks. These observers were unable to detect any ribonucleic acid in the inclusions. Sourander's careful study(3) of Negri bodies in dogs failed to reveal any nucleic acid. More recently, Sokolov and Vanag(4) claimed to have demonstrated presence of granules containing DNA and RNA in Negri bodies. Lépine and Atanasiu(5) found that Negri bodies in the brain were stained red by concentrated solutions of acridine orange in distilled water or in buffer at pH 7.0.

Inclusions produced in cells *in vitro* after infection with rabies virus could not, however, be readily distinguished from the intensely red-staining cytoplasm(5). Finally, Kissling and Reese(6) found round to irregular-shaped, dense reddish-orange masses in the cytoplasm of hamster kidney cells inoculated with a tissue culture-adapted strain of rabies virus, standard challenge virus (CVS) 11, and stained with acridine orange at pH 4. Fernandes *et al*(7) have adapted the CVS strain of rabies to several tissue culture systems, observing the formation of cytoplasmic inclusion bodies closely resembling Negri bodies which have provided good material for cytochemical study. The two rabies-infected tissue culture systems chosen for this study were the neonatal hamster kidney fibroblast line, BHK-21, clone 13 (BHK-21) where the number of cells showing the presence of cytoplasmic inclusions and size of the inclusions vary from cell generation to generation, and the rabbit endothelium (RE) line in which a true carrier state has been established with all cells containing inclusions of uniform size.

Materials and methods. Virus-infected cells. Monolayer tissue cultures of RE and BHK-21 cells(8) were propagated in milk dilution bottles and passed twice weekly. The medium consisted of modified Eagle's basal medium (9) supplemented with 10% millipore-filtered calf serum and containing 25 ml 5.6% sodium

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