aldehyde (30 min). The tissue pieces were post-fixed in 1% osmium tetroxide for 1 h and processed for Araldite-Epon embedding. Sections were stained with lead and uranyl acetate.

The collagenase treatment of the living tissue produced very striking changes in the ultrastructure of the matrix but no changes were apparent in the cells (Figures b and d). After the treatment with collagenase for 2 h the matrix was seen to consist of some fibres and occasional granules; in addition, the latter were also different in appearance from those in the control (Figures a and e). After the 4-hour treatment with collagenase the matrix showed no granules and there was a still further reduction in the number of the fibres (Figures d and f). The presence of some fibres, which presumably are collagenous in nature[2], after this treatment indicates the relative resistance of the polymeric collagen, as compared with the tropocollagen, to collagenase.

On the other hand, the collagenase treatment of the prefixed tissue did not noticeably affect the ultrastructural appearance of the matrix constituents, even at the periphery of the tissue piece (Figure c). This indicates that collagenase has practically no effect on collagen after glutaraldehyde fixation of cartilage, perhaps because of the formation of complexes between collagen and glutaraldehyde[5] or proteoglycans[6]. However, the increased electron transparency of the ground substance suggests that some of the tropocollagen (or its procollagen precursor) may have been removed by the enzyme treatment.

In view of the fact that collagenase is known to be highly selective in its action there are two possible explanations for the virtual absence of the electron dense granules in the micrographs (Figures e and f) of the tissue treated with collagenase before fixation. One, that collagen is a major constituent of the granules, and two, that it plays a definite role in the formation of the granules at the time of tissue fixation. But others[2,3,7,8] have concluded from their work involving both specific staining procedures[2] and extraction of unfixed and fixed cartilage by hyaluronidase, guanidium chloride, $CaCl_2$ and trypsin[3,8] that the electron dense granules are largely made up of proteoglycans, and that collagen, if any, is only a minor constituent of the granules.

We conclude that collagen plays a definite role in the formation of the granules at the time of tissue fixation and that the granules are, therefore, fixation artifacts. This hypothesis further explains the association of the granules with the collagen fibres in electron micrographs, and is consistent with the observation that the granules from fixed cartilage tissue can be removed by treatment with hyaluronidase or trypsin[3].

[1] Commonwealth Academic Staff Fellow. Permanent address: Department of Zoology, University of Poona, Poona 411007, India.
[2] A. Serafini-Fracassini and J. W. Smith, *The Structure and Biochemistry of Cartilage* (Churchill Livingstone, Edinburgh 1974).
[3] V. J. Matukas, B. J. Panner and J. L. Orbison, J. Cell Biol. *32*, 365 (1967).
[4] M. Takeichi, Expl. Cell Res. *68*, 88 (1971).
[5] M. A. Hayat, *Principles and Techniques of Electron Microscopy* (Van Nostrand Reinhold Co., New York 1970), vol. 1.
[6] F. S. Steven, J. Knott, D. S. Jackson and V. Podrazky, Biochim. biophys. Acta *188*, 307 (1969).
[7] I. Mandl, Science *169*, 1234 (1970).
[8] H. C. Anderson and S. W. Sajdera, J. Cell Biol. *49*, 650 (1971).

Protection by Methylcholanthrene Against Hepatic Carcinogenesis in Rats Ingesting N-4-(4′-Fluorobiphenyl)acetamide

M. D. Reuber

11014 Swansfield Road, Columbia (Maryland 21044, USA), 24 July 1975.

Summary. Methylcholanthrene protected against the development of carcinomas of the liver in Fischer rats ingesting N-4-(4′-fluorobiphenyl)acetamide.

N-2-fluorenylacetamide (2-FAA) induces tumors of the liver in rats[1–3]. Modification of the chemical structure by elimination of the methylene bridge to form N-(4-biphenyl)acetamide decreases carcinogenicity of the liver[4–6]. When the halogen, fluorine, is substituted in the 4′-position, i.e., N-4-(4′-fluorobiphenyl)acetamide (4′F-4-BAA), animals again develop tumors of the liver.

Methylcholanthrene (MCA) inhibits the development of carcinomas of the liver in male rats ingesting 2-FAA[7]. The present study was done to determine if MCA would also prevent the induction of carcinomas of the liver by 4′F-4BAA.

Methods. Inbred male Fischer strain (F-344) rats from the National Institutes of Health were used when they were 12 weeks of age and weighed 230 to 246 g. They were divided into 2 groups of 25 animals each. One group of rats received 4′-F-4BAA and the second group was given MC simultaneously with the 4′-F-4BAA.

The carcinogen was incorporated in Morris Diet No. 272[8]. 4′-F-4BAA was added in the amount of 0.04%; and MCA 0.033%. The two diets were fed ad libitum continuously for 36 weeks. Thereafter, the rats were given Purina laboratory pellets. 48 weeks after the start of the experiment animals that survived were killed by exsanguination. Complete autopsies were performed. Tissues were fixed in 10% formalin, sectioned and stained with hematoxylin and eosin.

Results. Animals in both groups gained weight for 36 weeks, at which time the weights remained constant. Rats, with rare exceptions, survived for the 48 weeks duration of the experiment.

[1] H. P. Morris, J. natn. Cancer Inst., Suppl. *15*, 1535 (1955).
[2] H. P. Morris, C. A. Velat and B. P. Wagner, J. natn. Cancer Inst *18*, 101 (1957).
[3] R. H. Wilson, F. DeEds and J. A. Cox, Cancer Res. *7*, 453 (1947).
[4] E. C. Miller and J. A. Miller, J. natn. Cancer Inst., Suppl. *15*, 1571 (1955).
[5] E. C. Miller, T. L. Fletcher, A. Margreth and J. A. Miller, Cancer Res. *22*, 1002 (1962).
[6] H. P. Morris, C. A. Velat and B. P. Wagner, J. natn. Cancer Inst. *24*, 149 (1960).
[7] T. Miyajil, L. I. Moszkowski, T. Senoo, M. Ogata, T. Oda, K. Kawai, Y. Sayama, H. Ishida and H. Matsuo, Gann *44*, 281 (1953).

Carcinomas of the liver were present in all 23 male rats ingesting 4'-F-4-BAA; whereas, the 25 male rats given 4'-F-4-BAA and MCA did not have hepatic lesions. Metastases to the lungs were present in 4 rats with hepatic carcinomas. Not only did the rats in the group given the two chemicals not develop hepatic carcinomas, but they also did not develop hyperplastic nodules, i.e., precursors of carcinomas. The histopathology of hyperplastic nodules and carcinomas of the liver has been described in detail previously[8].

Three of 23 rats given 4'-F-4-BAA in the diet also had primary renal carcinomas and 2 had hyperplastic nodules, which precede the development of renal carcinomas. Renal lesions were not present in animals ingesting both chemicals. The histopathology has been described elsewhere[9].

Discussion. MCA decreases the incidence of carcinomas of the liver in rats given 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB)[10]. The chemical protects against hepatic carcinogenesis and cirrhosis in rats ingesting 2-FAA or N-2-fluorenyldiacetamide[7,11]. The incidence of carcinomas of the liver is markedly reduced in rats given MCA and diethylnitrosamine simultaneously; however, it is only slightly decreased in rats receiving MCA and dimethylnitrosamine[12]. MCA also protects against hepatic carcinogenesis induced by 4'-F-4-BAA.

The mechanism by which MCA protects against hepatic carcinogenesis induced by 3'-Me-DAB or 2-FAA has been studied. MCA increases the activity of the enzymes concerned with the hydroxylation of aromatic amines and the reduction of azo bond linkage and the N-demethylation of amino azo dyes[13,14]. Presumably, MCA also increases the activity of the enzymes that hydroxylate 4'-F-4-BAA.

[8] H. I. FIRMINGER and M. D. REUBER, J. natn. Cancer Inst., USA *27*, 559 (1961).
[9] M. D. REUBER, Path. Microbiol. *41*, 253 (1974).
[10] H. L. RICHARDSON, A. R. STIER and E. BORSOS-NACHTNEBEL, Cancer Res. *12*, 356 (1952).
[11] M. D. REUBER, unpublished data.
[12] C. HOCH-LIGETI, M. F. ARGUS and J. G. ARCOS, J. natn. Cancer Inst. *40*, 535 (1968).
[13] J. A. MILLER, Cancer Res. *30*, 559 (1970).
[14] J. A. MILLER and E. C. MILLER, J. natn. Cancer Inst. *47*, 5 (1971).

Decreased Incidence of Renal Cell Carcinoma in Rats with One Kidney Ingesting N-4-(4'-Fluorobiphenyl)acetamide

M. D. REUBER

11014 Swansfield Road, Columbia (Maryland 21044, USA), 8 July 1975.

Summary. Buffalo strain male rats, 12 weeks of age, ingesting 0.04% N-4-(4'-fluorobiphenyl)acetamide in a semisynthetic diet with both kidneys intact were more susceptible to the development of hyperplasia and carcinomas of the kidney than were rats with a uninephrectomy.

The fluorinated biphenyl derivative of 2-acetylaminofluorene, N-4-(4'-fluorobiphenyl)acetamide (4'-F-4BAA), induces carcinomas of the kidney in rats[1–7]. Male rats are more susceptible to renal carcinogenesis than female rats and older rats are more susceptible than younger rats[8,9].

Since 4'-F-4BAA is not absorbed well from the gastrointestinal tract, much of the chemical remains in the feces of rats ingesting the diet[10]. It was felt that if the amount of target tissue, i.e. kidney, for the chemical to interact with was decreased, the incidence of carcinomas might be increased. Therefore, the incidence of renal lesions in intact animals ingesting 4'-F-4BAA was compared with that in animals with a uninephrectomy.

Methods. Inbred Buffalo strain male rats 12 weeks of age and weighing 266 to 281 g were used. There were 2 experimental groups of 20 rats each. The groups consisted of 1. intact male rats and 2. male rats with a left nephrectomy. Nephrectomies were performed 1 week before the start of the experiment.

The carcinogen was fed in Morris Diet No. 272[8]. 4'-F-4BAA was added in the amount of 0.04%. The carcinogen-containing diet was fed ad libitum continuously for 36 weeks. Thereafter, the rats were given Purina laboratory pellets. Animals were weighed every 2 weeks. 48 weeks after the start of the experiment all surviving animals were killed by exsanguination.

Complete necropsies were done at the time of death or killing of the animals. Tissues were fixed in 4% formaldehyde and stained routinely with hematoxylin and eosin, and when indicated, with the periodic acid-Schiff (PAS) technique.

The findings in the kidneys were classified as 1. no hyperplasia, 2. hyperplasia, 3. hyperplastic nodules, 4. small carcinomas (5 mm or less), and 5. large carcinomas (greater than 5 mm)[11].

Results. Animals of both groups steadily gained weight for 36 weeks, at which time the weights remained constant. Rats with a left nephrectomy survived for an average of 44 weeks (42 to 48); intact rats survived for 48 weeks. The number of animals with lesions of the kidney is shown in the Figure.

Carcinomas of the kidney were observed in 9 of 16 intact male rats; whereas 3 of 17 rats with the left kidney removed had renal cell carcinomas. The carcinomas in animals of the latter group were 5 mm or less in size. Hyperplastic renal nodules were present in 6 intact rats

[1] E. C. MILLER and J. A. MILLER, J. natn. Cancer Inst., Suppl. *15*, 1571 (1955).
[2] J. A. MILLER, R. B. SANDLIN, E. C. MILLER and H. P. RUSCH, Cancer Res. *15*, 188 (1955).
[3] E. C. MILLER, R. B. SANDLIN, J. A. MILLER and H. P. RUSCH, Cancer Res. *16*, 525 (1956).
[4] E. C. MILLER, T. L. FLETCHER, A. MARGRETH and J. A. MILLER, Cancer Res. *22*, 1002 (1962).
[5] H. P. MORRIS, J. natn. Cancer Inst., Suppl. *15*, 1535 (1955).
[6] H. P. MORRIS, C. A. VELAT and B. P. WAGNER, J. natn. Cancer Inst. *18*, 101 (1957).
[7] H. P. MORRIS, C. A. VELAT and B. P. WAGNER, J. natn. Cancer Inst. *24*, 149 (1960).
[8] M. D. REUBER, J. natn. Cancer Inst. *54*, 97 (1975).
[9] M. D. REUBER, Gann *65*, 389 (1974).
[10] H. N. DYER, personal communication.
[11] M. D. REUBER, Path. Microbiol. *41*, 253 (1974).