Serum Lipoproteins during Chemical Carcinogenesis

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1. The serum-protein and -lipoprotein patterns of rats, given a purified diet containing 0%, 0.005% or 0.03% 2-acetamidofluorene, have been determined by disk electrophoresis. 2. An abnormal high-density lipoprotein was detected from the first week of the experiment in the serum of the animals given the carcinogen. The low-density lipoproteins also increased on prolonged feeding with the carcinogen.

The role of lipids in chemical carcinogenesis is still a subject of considerable discussion (Haven & Bloor, 1956). Haven, Bloor & Randall (1951) have demonstrated large increases in rat serum lipids during the proliferation of preformed tumour cells. Barclay *et al.* (1962, 1964) have investigated the lipoprotein complexes in the serum of women with breast cancer and in the serum of rats bearing Walker carcinosarcoma 256. They have reported a large increase in the low-density lipoproteins in the serum of cancer subjects or tumour-bearing rats as compared with normal subjects or rats. However, no data are available in the literature on the serum lipoproteins of animals during various stages of tumour induction and growth.

Narayan, Narayan & Kummerow (1965) and Narayan, Creinin & Kummerow (1966a) developed a disk-electrophoresis method to determine the serum-lipoprotein patterns of human and rat serum. This method is unique in that a complete lipoprotein pattern representing all classes of lipoproteins can be obtained with as little as 10 μ l. of blood serum. The resolution obtained is superior to that observed with other electrophoretic or ultracentrifugal methods. In the present investigation, the serum protein and lipoprotein patterns of rats were determined at regular intervals by using this method during chemical carcinogenesis induced by 2-acetamidofluorene.

MATERIALS AND METHODS

Animals. Male rats (240; Holtzman Co., Madison, Wis., U.S.A.) weighing 50–60 g. were divided into three equal groups, A, B and C. Group A received a purified diet containing casein, 20.0%, cerelese, 66.1%, corn oil, 10.0%, Wesson salt, 3.5%, choline chloride, 0.3%, and all the necessary vitamins. Group B received the purified diet and 0.005% 2-acetamidofluorene (Eastman Organic Chemicals, Rochester, N.Y., U.S.A.), and group C received the purified diet and 0.03% 2-acetamidofluorene. The

2-acetamidofluorene was dissolved in the oil with the aid of a Pyro-Magnetstir (Labline Inc., Chicago, Ill., U.S.A.) before it was added to the basal diet. The rats were fed *ad libitum* once a day in the morning. Every 2–4 weeks, four rats were chosen at random from each group by drawing numbers without replacement. The rats were anaesthetized with Nembutal and blood was obtained from the abdominal aorta. The blood was taken in the afternoon from animals which had been fed the previous day but not on the day of collection of blood.

Electrophoretic and ultracentrifugal procedures. The protein and lipoprotein patterns in 3.75% polyacrylamide gel were obtained on individual serum samples with a model 12 disk electrophoresis equipment (Canalco, Rockville, Md., U.S.A.) according to procedures previously described (Narayan *et al.* 1966a; Narayan, Narayan & Kummerow, 1966c). The lipoproteins were stained with Sudan Black B and densitometer traces of the gel patterns were obtained by using a Canalco model E microdensitometer. Preparative ultracentrifugation was conducted in a Spinco model L ultracentrifuge by the method of Havel, Eder & Bragdon (1955) with a 40.3 rotor at solution densities of 1.063 and 1.21 g./ml. to obtain the total low-density and the total high-density rat serum lipoproteins.

RESULTS

Serum-lipoprotein patterns. Most of the results are presented as densitometer tracings of the serum-lipoprotein patterns obtained by disk electrophoresis (see, for example, Fig. 1). Albumin (peak 1) binds a small quantity of the Sudan Black B used to pre-stain the lipoproteins and appears after electrophoresis as either a light-green or brown band. By electrophoresis of lipoprotein fractions separated from normal rat serum by ultracentrifugation at different densities (see the Materials and Methods section), it was established that peak 2 represented the high-density lipoproteins and that the peaks 3, 4 and 5 represented the low-density lipoproteins (Narayan, Dudacek & Kummerow, 1966b).

Fig. 1. Serum-lipoprotein patterns of rats during the first week of the experiment. Densitometer setting: E 0-2-0; chart speed 1; gain 5. The arrows indicate the origin (main gel-spacer gel interface) and migration is from right to left. Each pattern is from the serum sample obtained from an individual rat. a, b, c, d, group A; e, f, g, h, group B; i, j, k, l, group C.

Fig. 2. Serum-lipoprotein patterns of rats during the third week of the experiment. a, b, c, d, group A; e, f, g, h, group B; i, j, k, l, group C. See legend to Fig. 1 for further details.

Subtle changes in the rat serum-lipoprotein patterns were evident during the first week of feeding with the carcinogen 2-acetamidofluorene. In the serum patterns of rats given 0.03% 2-acetamidofluorene (group C) a new peak (Fig. 1, peak 2a) was observed. This component appeared in the density fraction 1.063-1.21 and was therefore a high-density lipoprotein. During the third week, a dramatic increase in this component was observed

in the serum of rats from group C, and it also appeared as a small peak in two serum patterns of rats in group B. The areas of peaks 3 and 4 decreased in group C, whereas those of peak 5 remained unchanged (Fig. 2). During the fifth week, the areas of peaks 3 and 4 were considerably diminished in the lipoprotein patterns obtained from animals in groups B and C. Although the total high-density lipoprotein components increased during this week

in the serum of rats given 0.03% acetamidofluorene, the new high-density lipoprotein component (peak 2a) appeared to be unresolved from the principal high-density component (peak 2). In the serum samples of rats given 0.005% acetamidofluorene, increased concentrations of high-density lipoprotein were also found. During the seventh week, there were no significant differences between the serum-lipoprotein patterns of rats in the low-carcinogen group and those of the rats in the control group. However, in rats given 0.03% acetamidofluorene, the serum high-density lipoprotein (peaks 2 and 2a) were greatly elevated and, in two samples, the lipoprotein components represented by peaks 3 and 4 were totally absent. In the patterns of three samples from this group, peaks 2 and 2a were unresolved and appeared as one large peak. During the tenth week, the new high-density component (peak 2a) was present in high concentration in the serum of rats in group C and was well resolved from peak 2. The areas of peak 5 in the serum-lipoprotein patterns were greater in rats in group C than in rats in group A. The new high-density lipoprotein component was also present in small concentration in the serum of rats from group B.

During the fifteenth and eighteenth weeks, no significant differences were observed between the lipoprotein patterns of rats from groups B and A.

In group C, the high-density lipoprotein (peaks 2 and 2a) as well as the low-density lipoprotein (peak 5) concentrations were higher than in group A. During the twenty-second week, blood was collected from four rats from group A and from seven rats from group C. Although there were increased amounts of high-density lipoprotein and all other lipoproteins in several samples from group C, the striking differences in the patterns were in the areas of peak 5, which were much greater than the corresponding areas observed with samples from group A (Fig. 3). Group C was terminated after the twenty-sixth week as a result of many deaths, possibly due to degenerative changes in the liver and malignant growth.

Serum-protein patterns. No major differences occurred in the total serum-protein patterns among the rats in the three groups during the entire experimental period, possibly because of the high protein content of the diet. Densitometer tracings of the protein patterns during the third week of the experiment are typical of those obtained during the entire period and are shown in Fig. 4. In marked contrast with the lipoprotein patterns obtained during this week (Fig. 2), the protein patterns indicated little or no change due to the ingestion of the carcinogen.

Tissues. Gross examination of the tissues of rats killed at intervals indicated that tumours of the

Fig. 3. Serum-lipoprotein patterns of rats during the twenty-second week. a, b, group A; c, d, e, f, group C. Chart speed setting was 5. See legend to Fig. 1 for further details.

Fig. 4. Serum-protein patterns of rats during the third week of the experiment. a, b, group A; c, d, group B; e, f, group C. See legend to Fig. 1 for further details.

liver, mammary gland or ear duct were present in rats given 0.03% 2-acetamidofluorene during and after the tenth week, which is in accord with the results reported by Miller, Miller & Hartmann (1961). The early occurrence of tumours in the present experiments may be due to the use of young rats, which are known to form more of the *N*-hydroxy metabolite of 2-acetamidofluorene than adult rats (Margreth, Lotlikar, Miller & Miller, 1964). Among the rats in groups A and B, tumours were not detected during the experimental period. Histological examination of the tissues has confirmed these macroscopic observations. The liver tumours were of the trabecular type in most cases although some also showed anaplasia.

DISCUSSION

It is clear that major changes in the serum lipoproteins of rats occur during 2-acetamidofluorene-induced chemical carcinogenesis. These changes have been constantly observed in rats given a high dosage of 2-acetamidofluorene and have been noticed also in the patterns of some of the rats given a low dosage of 2-acetamidofluorene. During the preneoplastic state, i.e. before the tenth week, increased concentrations of high-density lipoprotein and of a new high-density lipoprotein component occur with the high dosage of 2-acetamidofluorene, whereas, during the neoplastic stage, both the high- and low-density components increase. Barclay *et al.* (1962) have observed high concentrations of low-density lipoproteins in the serum of

rats bearing Walker carcinosarcoma 256. However, these workers did not determine the high-density lipoproteins. In subjects with cancer, the serum concentration of high-density lipoprotein *S*₀₋₄ (1.125, sodium chloride) was observed to be low (Barclay *et al.* 1964). On the other hand, Rejnek, Bednarik, Rerabkova & Dolezal (1963) have reported the presence of an abnormal high-density lipoprotein species in the serum of cancer subjects and of pregnant women. This abnormal human serum high-density lipoprotein may be related to the new rat serum high-density lipoprotein observed in the present experiments.

Significant changes in the serum low-density lipoproteins have been demonstrated by a number of workers by variation of the nature of the dietary fat. However, dietary factors have not been shown to influence the high-density lipoproteins (Scanu, 1965). In the present experiments, it was not clear whether increased amounts of high-density lipoprotein were synthesized because they were required for the formation of tumour tissue or as a result of a toxic response by liver cells. In rats that have been given carbon tetrachloride or ethionine, decreases in both the low- and high-density lipoproteins have been reported (Lombardi & Ugazio, 1965; Ugazio & Lombardi, 1965). Therefore the changes observed in the lipoprotein patterns of rats given 2-acetamidofluorene were probably not directly related to the toxic response elicited by the carcinogen. The elevation in the concentrations of lipoproteins observed here may also be explained on the basis of mobilization of lipids from the fat

depots. However, the need for mobilization, and, particularly of this unusual kind, at a very early stage of 2-acetamidofluorene ingestion, is unclear. Nichols, Rehnberg, Lindgren & Wills (1962) have reported increases in the low-density lipoprotein as well as in the high-density lipoprotein on administration of vegetable-oil emulsions to human subjects. However, in the rat, oil ingestion failed to produce significant changes in the concentrations of high-density or low-density lipoproteins, as determined by the disk-electrophoresis method (K. A. Narayan, unpublished work).

It has been suggested that tumours have a tendency to draw on the host for their supply of phospholipids and essential fatty acids (Haven *et al.* 1951). The high-density lipoproteins have a high concentration of phospholipids on a protein-free basis (Scanu, 1965) and thus it is possible that certain cellular mechanisms are triggered to increase the synthesis and secretion of these lipoproteins, so as to provide a favourable environment for neoplastic transformation and growth. In this connexion, the principal lipoproteins of cellular fractions of rat liver have been shown to be high-density lipoproteins of high molecular weight (Napier & Olson, 1965). Whether the new high-density lipoprotein component observed in the serum of rats given 2-acetamidofluorene represents an essential structural component of tumour cells remains for speculation and further study.

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REFERENCES

- Barclay, M., Calathes, D. N., Garfinkel, E., Terebus-Kekish, O., Barclay, R. K. & Skipski, V. P. (1962). *Arch. Biochem. Biophys.* **98**, 391.
- Barclay, M., Escher, G. C., Kaufman, R. J., Terebus-Kekish, O., Greene, E. M. & Skipski, V. P. (1964). *Clin. chim. Acta*, **10**, 39.
- Havel, R. J., Eder, H. A. & Bragdon, J. H. (1955). *J. clin. Invest.* **34**, 1345.
- Haven, F. L. & Bloor, W. R. (1956). *Advanc. Cancer Res.* **4**, 237.
- Haven, F. L., Bloor, W. R. & Randall, C. (1951). *Cancer Res.* **11**, 619.
- Lombardi, B. & Ugazio, G. (1965). *J. Lipid Res.* **6**, 498.
- Margreth, A., Lotlikar, P. D., Miller, E. C. & Miller, J. A. (1964). *Cancer Res.* **24**, 920.
- Miller, E. C., Miller, J. A. & Hartmann, H. A. (1961). *Cancer Res.* **21**, 815.
- Napier, E. A., jun. & Olson, R. E. (1965). *J. biol. Chem.* **240**, 4244.
- Narayan, K. A., Creinin, H. L. & Kummerow, F. A. (1966a). *J. Lipid Res.* **7**, 150.
- Narayan, K. A., Dudacek, W. E. & Kummerow, F. A. (1966b). *Biochim. biophys. Acta*, **125**, 581.
- Narayan, K. A., Narayan, S. & Kummerow, F. A. (1965). *Nature, Lond.*, **205**, 246.
- Narayan, K. A., Narayan, S. & Kummerow, F. A. (1966c). *Clin. chim. Acta*, **14**, 227.
- Nichols, A. V., Rehnberg, C. S., Lindgren, F. T. & Wills, R. D. (1962). *J. Lipid Res.* **3**, 320.
- Rejnek, J., Bednarik, T., Rerabkova, E. & Dolezal, A. (1963). *Clin. chim. Acta*, **8**, 108.
- Scanu, A. M. (1965). *Advanc. Lipid Res.* **3**, 63.
- Ugazio, G. & Lombardi, B. (1965). *Lab. Invest.* **14**, 711.