MOBILIZATION OF BASOPHILE SUBSTANCE (RIBONUCLEIC ACID) IN THE CYTOPLASM OF LIVER CELLS WITH THE PRODUCTION OF TUMORS BY BUTTER YELLOW*

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In a foregoing study (2) recognizable lesions of the parenchymatous cells of the liver and of newly formed bile ducts with none of the characteristics of neoplasm were found to precede the development of tumors derived from these structures. Focal hyperplasia was a precursor of trabecular or of adenomatous hepatomas; with it the cytoplasm of liver cells took a deeply basophile stain referable perhaps to the presence of ribonucleic acid and active division of liver cells by mitosis was in progress. In some instances newly formed bile ducts underwent cystic dilatation and from them cystadenomas developed. In other animals there was dilatation of newly formed bile ducts with acute inflammation and proliferation of fibrous tissue producing microscopically recognizable lesions conveniently designated cholangiofibrosis. This lesion was the precursor of tumors that had the characteristics of cholangiomas. All of these tumors might become malignant and form metastases.

The present study has been undertaken to obtain information concerning the behavior of basophile material of the liver during the progress of the changes which, following the administration of dimethylaminoazobenzene (butter yellow) to white rats, result in focal hyperplasia and the formation of tumors.

The presence of a substance that stained with carmine was observed by R. Heidenhain (3) in the basal part of cells of the pancreas and stomach, during the course of his well known studies of the changes accompanying secretion. The wide distribution of cytoplasmic material that had staining characters of the nucleus was later recognized and Garnier (4) proposed for it the name "ergastoplasm" but it has been more frequently designated basophile or chromaphile material. Some regarded it as an artificial product, the result of fixation, and others suggested that it represented poorly fixed mitochondria.

The substance that constitutes the Nissl bodies of nerve cells resembles in its staining characters the basophile substance which is found widely in other tissues. The early studies of Held (5) failed to demonstrate Nissl bodies in fresh cells and he reached the conclusion that they were formed by fixing agents such as alcohol or bichloride of mercury but especially by the action of acids and suggested that they might be formed by changes following death of cells.

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Bensley and Gersh (6) examined Nissl bodies in tissue frozen, dried in a vacuum, and embedded directly in paraffin and expressed the opinion that the Nissl substance was not distributed uniformly through the cytoplasm but actually occurred as aggregates of fine particles of which the form may be somewhat modified by different fixing agents. In liver cells of the salamander Bensley and Gersh believed that they had succeeded in staining by appropriate methods chromophile bodies and mitochondria in the same cell. It is noteworthy that the chromophile substance of the liver cells was accurately pictured in the comprehensive treatise of Pfuhl (7) on the cytology of the liver but this material was not distinguished from mitochondria and the terms chondriosomes, plasmosomes, and mitochondria were applied to it. Pfuhl stated that plasmosomes often encircled the nucleus in a zone between nucleus and the periphery of the cell. In places they were found more numerous and rod-like near the margins of the cell columns and here might form two parallel rows.

The observations of Caspersson (8) and later of Brachet (9) and others have demonstrated that basophile substance with the characteristics of ribonucleic acid occurs in the cytoplasm of many cells. The present study shows that this basophile material undergoes an orderly series of changes with the injury, regeneration, and tumor formation caused by butter yellow. In a later publication (10) procedures which show that these changes represent mobilization of ribonucleic acid in the cytoplasm will be discussed.

Roskin and Kharlova (11) noted the basophile stain of the cytoplasm of cells from a variety of human tumors and find that it disappears on treatment with ribonuclease. Caspersson and Santesson (12) using ultraviolet photographs and a few absorption spectra studied different kinds of epithelial tumors and found two types of cells. One is small with, they state, abundant ribonucleotides and high concentration of protein and seems to represent the actively growing part of the tumor. The other type is large with a large nucleus and contains no ribonucleotides and scant concentration of protein.

Methods

Fixation of the basophile substance of the cytoplasm is doubtless dependent upon factors that influence the solubility or precipitation of nucleoprotein and of nucleic acid. Penetration of the fixing fluid is essential and with thick blocks of tissue fixation is limited to a narrow zone below the surfaces, whereas in the deeper part the basophile material has disappeared. Tissue has been cut with a razor blade in pieces 1 mm. or less in thickness and fixed in Zenker's fluid to which 5 per cent of acetic acid has been added. The addition of acetic acid is not necessary but evidently aids penetration of mercuric bichloride. A less satisfactory agent is a mixture of 80 per cent alcohol (90 cc.), 10 per cent formalin (5 cc.), and acetic acid (5 cc.). Absolute alcohol is a good fixing agent for the basophile material but it penetrates poorly. Excellent preparations are obtained with the Giemsa stain which contains Azur II and Azur II eosin.

Pyronin stains well the basophile material of the cytoplasm after fixation with Zenker's fluid containing acetic acid, with the mixture of alcohol, formalin, and acetic acid, and with absolute alcohol. The staining method of Pappenheim in which pyronin and methyl green are used is satisfactory. Fixation with Zenker's fluid to which formalin has been added instead of acetic acid, though recommended, has given uncertain results. Staining with pyronin has special interest because the claim has been made (Berg, 13), that it demonstrates the presence of stored protein in liver cells.

Formalin (10 per cent of formalin or approximately 4 per cent of formaldehyde) penetrates readily and fixes the basophile material very effectively. The Giemsa method after formalin fixation stains deeply the diffusely distributed basophile substance of the cytoplasm but stains as well somewhat less deeply the cytoplasm which with other methods of fixation is acidophile. Nevertheless the results of staining after formalin fixation are not uniform and some solution of nucleic acid may occur after the tissue has been fixed.

In some instances the liver has been perfused with lanthanum acetate (M/5) or has been immersed in a solution of the same concentration in order to precipitate nucleic acid so that after fixation in the mixture of alcohol, formalin, and acetic acid or in alcohol alone it may be stained by basic dyes.

Mitochondria have been fixed by a solution of potassium bichromate and formalin (Regaud's fluid) and stained with anilin fuchsin or hematoxylin but unfortunately with this method the basophile material of the cytoplasm is not fixed so that it can be stained well by basic dyes.

Changes in the liver caused by butter yellow and the influence of diet on them have been described in earlier publications (1, 2) and the observations made during the progress of these experiments have been used to supplement and confirm those that will be described here. The diets used in the present experiments have been used in those that have preceded and their influence on the production of tumors and of cirrhosis has been essentially the same in both.

Nomenclature

The nomenclature used in this publication is that previously described (2). The portal unit like the lobule of other glands is the parenchyma that is tributary to a primary branch of the bile duct. A primary portal space with its bile duct, portal vein, and hepatic artery enters each portal unit. The terminal branches of the hepatic vein, that is, the central veins are situated at the periphery of the portal unit and are united by the so called vascular septa. It is doubtless desirable, though somewhat unfortunate, in accordance with accepted usage, to designate as the hepatic lobule the parenchyma that surrounds the central vein and has portal spaces at its periphery. The hepatic unit is a unit of secretory activity whereas the hepatic lobule may be regarded as a vascular unit of structure. Primary portal spaces carrying primary branches of the three contained structures into the hepatic units should be distinguished from the secondary portal spaces which have significant characteristics.

Cirrhosis as in a preceding publication (2) has been designated incipient, early, and nodular in accordance with the arbitrary definitions assigned to these terms.

EXPERIMENTS

White rats have been given dimethylaminoazobenzene (butter yellow) mixed with their diet in the proportion of 0.06 per cent. In one group of experiments the effects of two diets used in preceding experiments (1) have been compared. One diet (E, Table I) has a high protein content, representing 41 per cent of its caloric value, and the other (F, Table II) a low protein content, representing 7 per cent of its caloric value. The fate of these animals is shown in Tables I and II. Some animals have died following the administration of butter yellow, the greater number have been killed at selected intervals, and a few have been killed because they have seemed to be moribund.

The weight of the liver, given in Tables I and II as per cent of the body

weight, is a rough index of its enlargement as the result of hyperplasia or of tumor formation. In general terms weights of approximately 6 per cent suggest hyperplasia, whereas weights in excess of this figure occur in association with grossly recognizable tumors. The number of mitotic figures in liver tissue

TABLE I

Animals That Received Diet E with High Protein Content

No. of animal	Time of butter yellow administration	Mode of death	Per cent of liver weight to body weight	Fatty degeneration	Cirrhosis	Mitoses per 50 fields	Chromatolysis	Focal hyperplasia	Cystic ducts	Cholangiofibrosis	Hepatomas	Cholangiomas
	days											
1	17	D										
2	34	K	4.5	+	Incipient	0	+					
3	35	K	4.8			0	+					
4	60	K	5.2			0	+					
5	60	K	5.7			0	+					
6	84	K				0	+					
7	84	K	4.9		Incipient	1	+	±				
8	123	K			Incipient	7	+	+				
9	123	D										
10	140	D										
11	150	K	6.1		Early	33		+	+	+	+	
12	150	K	5.3		Incipient	0	+	±				
13	160	D										
14	181	K	6.1		Early	4	+	+				
15	182	K	4.7		Incipient	0	+	±				
16	193	SK	6.9		Early	0	+	±				
17	210	K	6.6		Incipient	8	+				+	
18	213	K	5.5		Incipient	1	+	+				
19	237	SK	5.5		Early							
20	244	K	6.9		Incipient	1	+	±				
21	244	K	6.3		Incipient	3	+	+			+	
22	253	SK			Early	1	+	+	+		+	

The mode of death is indicated by D, died, K, killed with ether, and SK, sick and killed. Data concerning animals that have died have been omitted. The severity of cirrhosis is indicated by the terms, incipient, early, and nodular. + indicates that a lesion has been advanced; ±, that it has been slight.

containing no tumor was counted in 50 high power fields in sections 5 microns in thickness.[1] It is an approximate index of their frequency for little variation has been found when this count has been doubled. Mitotic figures are seldom found in the normal liver and it will be shown occur under abnormal conditions

[1] I am indebted to Mrs. James Alexander (Miss M. L. Muckley) for a large part of these counts.

in foci characterized by basophilia of the cytoplasm of parenchymatous cells. The number of mitoses serves as an index of the activity of hyperplasia at the time of observation.

The changes in the liver of animals that have received butter yellow in association with a diet (E) containing abundant protein have differed widely from those in animals that have received a diet (F) with low protein content. With diet E the body weight of animals has increased, whereas, with diet F body

TABLE II

Animals That Received Diet F with Low Protein Content

No. of animals	Time of butter yellow administration	Mode of death	Per cent of liver weight to body weight	Fatty degeneration	Cirrhosis	Mitoses per 50 fields	Chromatolysis	Focal hyperphasia	Cystic ducts	Cholangiofibrosis	Hepatomas	Cholangiomas
	days											
23	34	K	5.1	+		2	+					
24	36	K	4.2	+		0	+					
25	42	K	6.5	+	Early	6	+					
26	55	K			Early	0	+					
27	61	K	4.7	+	Early	4	+					
28	86	K	4.1	+	Early	4	+					
29	92	D										
30	114	D										
31	123	K	5.3	+	Nodular	5	+			+		
32	127	D										
33	134	K	4.5	+	Early	13	±					
34	151	K	4.1	+	Nodular	11	±					
35	151	K	5.1	+	Early	6	+			+		
36	178	D										
37	182	K	8.9	+	Nodular		+	+	+	+	+	
38	183	K			Nodular	23	+	+	+	+		+
39	190	D										
40	211	K	13.5	+	Early	26		+	+	+	+	+
41	214	K	14.5	+	Nodular	1	+	+	+	+	+	+
42	245	K	7.3	+	Early	5	+	+	+			+

weight has been maintained but not increased. Within the period of the experiment there has been no significant difference in mortality in the two groups. (See Tables I and II.)

In animals that have received the high protein diet (Table I) the most obvious change that has occurred in the first 3 months of butter yellow administration is diminution of the basophile material of the cytoplasm of liver cells. In accordance with accepted nomenclature with reference to the Nissl bodies of nerve cells the term chromatolysis will be used to designate this change which may occur in association with degenerative processes so profound that they end

with the death of the cell. Nevertheless, in animals on the high protein diet there is scant reaction during the first 3 months with no mitotic division of liver cells and scant evidence of cirrhosis. After 4 months active mitotic division of liver cells has become evident, there is accumulation of basophile material in liver cells and the earliest stages of cirrhosis are seen. Tumor formation has been observed 5 months after the beginning of butter yellow administration but tumors have been found in only a small part of the animals and have been in all instances hepatomas.

In animals that have received a diet with low protein content (Table II) parenchymatous changes have occurred promptly and are more severe than with the high protein diet. Widespread fatty degeneration, evident 1 month after the beginning of butter yellow administration, has been present throughout the course of the experiment, diminishing in extent after 5 months. Basophile material of the cytoplasm is much decreased with this fatty degeneration and disappears completely in the peripheral part of the hepatic unit, that is, in contact with the terminal branches of the hepatic vein (central veins), but does not disappear from the parenchymatous cells about the portal spaces. Regeneration of parenchymatous cells occurs promptly and cells in mitotic division are readily found in the early months of butter yellow administration. It is noteworthy that none have been found during the corresponding period in animals that have received diet E (see Table I). Nevertheless, the restoration of the basophilia of the cytoplasm does not occur more rapidly in animals on the low than in those on the high protein diet. With the former, cirrhosis appears earlier and becomes more severe, being often nodular. With the low protein diet cystic ducts and cholangiofibrosis are usually found after 4 months and in contrast with the high protein diet cholangiomas appear in a considerable part of the animals.

Chromatolysis

In the normal liver with appropriate staining (Fig. 1) irregularly formed coarse basophile particles occur in the liver cells and are larger and more conspicuous as the central vein is approached than about portal spaces. In places basophile particles may have an elongated form and occur with a palisade-like arrangement in two rows near the two margins of the liver cell columns. The cytoplasm of liver cells about the portal spaces in a zone representing from one-fourth to one-third of the radius of the portal unit has a deeper basophile stain than that of the remaining parenchma and this deeper stain is associated with a diffuse basophilia of the cytoplasm not evident elsewhere.

In the part of the portal unit that surrounds the portal space and takes a diffuse basophile stain oval spaces throughout the cytoplasm are surrounded by delicate rims of basophile material much more lightly stained than those that form the basophile particles but in the neighborhood of the central veins with clumping of the elementary basophile bodies the cytoplasm elsewhere in

the cell loses its diffuse basophile stain (blue with Giemsa stain) and stains only with the acid dye (pink with the Giemsa stain). Nevertheless the structure of the cytoplasm is still recognizable and clear oval spaces are surrounded by rims of acidophile material. Comparison with cells stained by the usual methods for demonstration of mitochondria shows that the Giemsa stain defines these bodies, outlined by a rim of material which in one part of the hepatic unit is stained by the basic and in another by the acid dye.

After 1 or 2 months of butter yellow administration there is evident diminution of the basophile material of the liver cells, designated chromatolysis (Fig. 2). In the greater part of the portal unit toward the central vein basophile bodies are less numerous than in the normal liver and stain less deeply. They diminish as the vein is approached and immediately about it may be wholly absent. They are in large part aggregated about the nucleus so that the peripheral part of the cell is almost free from them (Fig. 10).

With diminution of the basophile material it becomes evident that the coarse particles are made up of minute bodies with clear oval space surrounded by a well defined rim of basophile material. To distinguish these small bodies from the larger particles they may be designated basophile bodies. They are longer than broad and usually pointed at one or both ends. Their relation to the cell nucleus is suggestive of some kind of attraction for those at a distance are often disposed with their long diameter in the direction of the nucleus, whereas, those in contact with it are closely applied with their long axis parallel to the nuclear membrane. In the coarse particles that are seen in normal cells basophile material fills the interstices between mitochondria.

After 2 months of butter yellow administration, more conspicuously later, a second form of chromatolysis occurs chiefly in the portal part of the hepatic unit (Figs. 11 and 12). Liver cells become swollen and rounded and finally separate from adjacent cells so that the continuity of the liver cell column is broken. Basophile bodies disappear completely from the central part of the cell surrounding the nucleus and remain only in a narrow zone at the periphery where they are disposed parallel with the circumference of the cell. The cytoplasm in the part of the cell that has no basophile material takes a bright pink color and here is seen the usual arrangement of oval mitochondrial spaces surrounded by a rim of acidophile material. With increasing size of these swollen cells several changes occur. The central part becomes finely vacuolated and ultimately vacuoles in large part or completely replace the mitochondria. At the periphery of the cell basophile bodies become paler and in many cells disappear completely. This degenerative change doubtless ends with the death of the cell, for the cell nucleus may lose its chromatin, become irregular in outline, and disappear. When this vacuolar degeneration and necrosis is widespread about portal spaces there is multiplication of cells of the fibrous tissue (incipient cirrhosis).

The chromatolysis, just described, may occur in well defined rounded foci

with a diameter approximately a third or a fourth of the radius of the portal unit. In most of the cells in these foci chromatolysis is far advanced so that many of them have lost all basophilia and some of them their nucleus as well.

Chromatogenesis; Focal Basophile Hyperplasia

After 4 months of butter yellow administration conspicuous new formation of basophile material is seen in association with the advanced chromatolysis that has occurred. Chromatogenesis which has been feebly evident at an earlier stage may now become well established and reaches an intensity in spots considerably in excess of that seen in the normal liver. Its distribution is focal because it occurs about a primary portal space or in an isolated area beside a primary or secondary portal space that is elsewhere surrounded by liver cells undergoing the degenerative changes that are associated with chromatolysis. Two features characterize these foci: there is diffuse basophile stain of the cytoplasm caused by uniform basophilia of the mitochondrial rims surrounding clear oval spaces; active division of cells by mitosis occurs in these foci and is infrequent elsewhere.

The cytoplasm of cells in mitosis is quite uniform and has a diffuse basophile stain (Fig. 7). With magnification of approximately 1000 diameters mitochondria with oval space and basophile rim are well seen. These appear to be somewhat smaller than those of resting cells. Fine basophile particles are recognizable but their relation to the mitochondrial rims is not definable for in places they seem to form part of the rim near one pole of the oval space. The basophile material of the mitochondria stains far less deeply than the chromosomes of the nucleus.

Two kinds of focal basophile hyperplasia are seen. (1) One consists of cells with diffusely stained cytoplasm like that of cells in immediate contact with portal spaces in the normal liver (Fig. 3). The intensity of the basophilia is greater than that of the normal cells and the foci, which vary in size, are in sharp contrast with the parenchyma elsewhere which is the site of chromatolysis. The usual architecture of the liver parenchyma is preserved and liver cell columns are formed. Changes that occur in the normal liver cells may be reproduced in these foci. At an increasing distance from the portal space they may contain scattered basophile bodies, these may form clumps and when clumping occurs the cytoplasm loses its diffuse stain. (2) A second variety of focal basophile hyperplasia is similarly characterized by diffuse staining of cytoplasm and mitotic division of cells, but liver cell columns acquire a tubular form (see Fig. 4). The cytoplasm of cells close to a portal space is diffusely and evenly stained but nearby liver cells are arranged about a lumen, readily seen in both longitudinal and cross-section. The part of the cell in contact with the lumen is much less deeply basophile than the basal half in which the nucleus is situated. Mitochondrial spaces may be seen arranged in rows at right angles

to the bases of the cells and delicate lines of deeply basophile material may lie between the rows. One or several spaces may be surrounded by basophile substance elongated in the same direction. At a greater distance from the portal space basophile bodies may be well formed and have a palisade-like arrangement near the margins of the liver cell columns. With this change diffuse stain of cytoplasm is absent and a lumen is not recognizable, but the sequence of these events is not evident.

Foci of tubular basophile hyperplasia are usually more extensive than those in which the columnar arrangement of liver cells is preserved. The latter are often conspicuous immediately below the surface of the liver where they surround terminal portal spaces (terminal bile ducts) and in many instances doubtless represent new portal units in process of formation.

The combination of chromatolysis and basophile hyperplasia is associated with a change which seems to be the result of injury to cells in foci of regeneration. Following butter yellow administration an occasional liver cell may acquire unusual size with a diameter several times that of a normal liver cell (Fig. 13). The nucleus of the cell is greatly enlarged and hyperchromatic with deeply stained nuclear membrane and one or several large nucleoli. The change which occurs in association with chromatolysis affects cells in the neighborhood of the portal space. Sharply defined basophile bodies may be scattered with no clumping in the cytoplasm about the nucleus and in the periphery of the swollen cell acidophile mitochondria with rim and central clear space are well separated from one another. In some foci of basophile hyperplasia single cells or groups of cells with diffusely basophile cytoplasm may undergo a similar change (Fig. 14). Mitochondria in the swollen cell may be separated and well defined. Those about the nucleus may have a rim stained by the basic dye whereas those in the periphery of the cell have a rim stained by the acid dye.

Development of Hepatomas from Foci of Basophile Hyperplasia

Trabecular hepatomas are characterized by broad anastomosing columns of cells much wider and more irregularly disposed than those of the normal liver. The tumor cells are usually much larger than liver cells and the nuclei are larger and with larger nucleoli but there is far greater variation in the size of nuclei than with normal cells. A distinctive feature of the tumor cell is the diffuse basophile stain of its cytoplasm which resembles that of cells in mitosis (Figs. 8 and 9). Mitochondrial spaces are surrounded by basophile rims and minute basophile particles give a stippled appearance to the cells. The cytoplasm in spite of the absence of deeply stained basophile bodies has basophilia much in excess of that of surrounding liver parenchyma and less than that often seen with basophile hyperplasia. In the early stage of tumor production mitotic division may proceed with astonishing activity. In rat 11 (Table I) killed 5 months after the beginning of butter yellow administration the liver paren-

chyma contained an unusually large number of mitotic figures, namely 0.66 per high power field, but in a trabecular hepatoma 6 mm. in diameter within this liver the number of mitoses per high power field was 8.9. In association with another small trabecular hepatoma (rat 41, Table II) the average number of mitoses in the parenchyma in general averaged 0.29 per high power field whereas in the tumor itself the average was 17 per field.

When trabecular hepatomas are in early stages of formation foci of basophile hyperplasia are abundant in the same liver. Not infrequently it is possible to follow the changes from liver cell columns with basophile hyperplasia to the broad irregular trabecula of a small hepatoma. The association of an early trabecular hepatoma with a focus of deeply basophile parenchyma is shown in Fig. 10 of a preceding publication (1). Here at the lower part of the microscopic tumor nodule narrow basophile columns of liver cells are in continuity with the broad trabecula of the hepatoma. These cells and the trabecula they form are identical with those of macroscopic tumor nodules in the same liver. This association of early hepatomas with foci of basophile hyperplasia has been found repeatedly in the present and in the earlier experiments and is more readily observed because tumor production by butter yellow is multicentric so that different stages in the production of tumors are often found in the same liver.

There is an intimate relation between hepatomas that are trabecular with solid columns of cells and those that are adenomatous with similar cells arranged about a lumen. A tumor may be trabecular in one part and adenomatous in another. Small adenohepatomas have usually occurred in association with widespread foci of tubular hyperplasia and the continuity of one with the other is often observed (Fig. 4). In passing from hyperplasia to neoplasm the cells increase in size and the tumor aveoli become much wider than the hyperplastic tubules. The nuclei are large and vary in size like those of trabecular hepatomas. The adenomatous alveoli have conspicuous lumina, in places widely dilated and irregularly formed. The cells about the lumen may be polygonal, cubical, or approximately columnar. The cytoplasm of these cells is in general diffusely basophile and stippled but the basal half in which the nucleus is situated is usually more deeply stained than that next the lumen and in this respect resembles that of cells in foci of tubular hyperplasia. Furthermore as with tubular hyperplasia mitochondrial spaces are oriented in rows perpendicular to the base of the cell and accentuation of basophile stain about single spaces or rows of spaces produces linear markings in the same direction.

Development of Cholangiomas from Basophile Hyperplastic Ducts of Cholangiofibrosis

The duct-like structures that constitute the distinctive feature of cholangiofibrosis are composed of low cubical cells about a round, often dilated lumen.

They undergo hyperplasia in places (Fig. 5) characterized by increase in size, columnar form, deep basophilia of cytoplasm, and active multiplication as shown by numerous mitotic figures. High columnar basophile cells may be found on one side of the lumen of a duct-like tubule whereas the cells of the remainder of the circumference are cubical and palely stained. Elsewhere columnar cells may completely surround the lumen which is then enlarged and irregular in shape. In the cytoplasm of these basophile cells clear mitochondrial spaces are surrounded by rims of basophile material and small basophile particles are recognizable. The mitochondrial spaces in high columnar basophile cells are usually arranged in rows parallel with the long axis of the cell and hence perpendicular to its base. This arrangement is best seen in the apical part of the cell because the base is almost completely occupied by the nucleus.

The newly formed ducts that have undergone basophile hyperplasia are conspicuously defined in ultraviolet photographs (see Fig. 6 in the following publication, 10) and evidently absorb almost completely ultraviolet radiation of wave length 2537Å. Furthermore the basophile material is removed by ribonuclease though the cell nuclei are unaffected by the enzyme (10).

In places the transition from the basophile hyperplastic ducts of adenofibrosis to cholangioma is clearly recognizable. An alveolus may have regularly formed basophile columnar cells on one side and a solid mass of polygonal cells on the other (Fig. 6). With transition from hyperplastic cells into solid masses of tumor cells basophilia diminishes but proliferating tumor cells are stained more deeply than the adjacent liver cells.

DISCUSSION

In association with a diet containing abundant protein and adequate in caloric value, mineral salts, and vitamins, dimethylaminoazobenzene (butter yellow) causes progressive injury of liver cells. Regeneration of parenchymatous cells proceeds slowly and cirrhosis with new formation of bile ducts progresses gradually and is never far advanced. After about 5 months hepatomas begin to develop from the parenchymatous cells of the organ. Diminution of the protein level of the diet lowers the threshold at which butter yellow causes injury of liver cells. There is widespread accumulation of visible fat within liver cells, prompt regenerative changes affecting both parenchymatous and newly formed bile ducts, and rapidly developing cirrhosis. The tumors that appear are not only hepatomas from the liver cells, but in much greater number than with the high protein diet, cholangiomas derived from newly formed bile ducts.

Following the administration of butter yellow there is, preceding the development of hepatomas, a sequence of changes in the basophile substance of the cytoplasm characterized first by diminution (chromatolysis) and later by

reaccumulation of this substance (chromatogenesis) beginning about portal spaces. Reaccumulation of basophile material is coincident with division by mitosis of liver cells. Hepatomas develop from foci of basophile hyperplasia.

Degeneration of liver cells injured by butter yellow reveals some aspects of the constitution of cytoplasm not otherwise readily demonstrable. The smallest basophile body of normal liver cells is an elongated structure consisting of basophile material surrounding a clear space of the size and shape of a mitochondrium. These bodies may clump together to form larger masses or may assume a palisade-like arrangement in parallel rows near the margins of a liver cell column. The basophile material like ribonucleic acid absorbs ultraviolet radiation and undergoes disintegration when treated with ribonuclease (10).

With degenerative changes caused by butter yellow basophile bodies may lose the intensity of their stain, diminish in number, and with death of the cell disappear completely. In animals that have received butter yellow in association with a low protein diet there is widespread fat accumulation in liver cells and disappearance of basophile bodies in zones about the central veins, representing more than half of the parenchyma. When butter yellow is given with a high protein diet chromatolysis proceeds gradually and basophile bodies that remain may form more or less compact clumps about the nuclei. This change affects a large part of the parenchyma in proximity to the central veins. About portal spaces on the contrary basophile bodies may disappear from the interior of cells, here later occupied by vacuoles, so that they persist only in a narrow margin at the periphery.

In appropriately stained tissue (Giemsa stain) undergoing the degenerative changes that have been cited, the relation of basophile substance to the mitochondria is often evident, particularly when they are separated from one another presumably by the accumulation of fluid in the cell (Fig. 14). In foci of hyperplasia mitochondria consist of a narrow rim of material with a dull basophile stain surrounding a clear space which is usually oval. This basophile material stains much less intensely than chromatin. In addition fine particles are recognizable and appear to give a stippled appearance to the cytoplasm, well seen in cells in mitosis. These particles are just within the range of visibility and their relation to the basophile material of the mitochondria is uncertain. It is possible that they are in part the microsomes that Claude (14, 15) obtained by high speed centrifugalization, though presumably they are larger than microsomes which are usually not within the range of visibility. With chromatolysis basophile material disappears from the rim of the mitochondrium and this rim then stains with acid dyes. It is noteworthy that with clumping of basophile bodies mitochondria are identifiable in the cytoplasm about the clumps by their acidophile stain (pink by the Giemsa method).

When basophile bodies are in process of formation basophile rims about what seem to be mitochondrial spaces have become deeply basophile and stain with

the intensity of chromatin but the accumulation of basophile material forms a body which is considerably larger than a mitochondrium and has one or both ends pointed. Coarse clumps of basophile material are formed in which the component basophile bodies are only vaguely distinguishable but when chromatolysis brings about diminution of basophile substance it is evident that these clumps are made up of small vesicle-like bodies, for they lose their coarse irregular form and are seen as delicate but sharply defined oval rims about clear oval spaces. The probability that the basophile bodies are formed by increase of basophile material at the periphery of mitochondria is supported by the observation that acidophile mitochondria of uniform size persist in a cell in which advanced chromatolysis has brought about complete disappearance of basophile material.

Two forms of basophile hyperplasia have been observed and both are characterized by cells with basophile mitochondria which give a diffuse stain to the cytoplasm. In one liver cell columns are still maintained and in the other the columns have taken on a tubular form so that lumina are recognizable within them. In the former there is no evident orientation of mitochondria but in the latter mitochondria are found in rows at right angles to the bases of the component cells and linear basophile markings may be seen between or about them.

With the transformation of basophile hyperplasia into tumor the cells retain their diffuse cytoplasmic stain and mitochondria have the rim of basophile material that is well defined in cells in mitosis. As in the latter fine particles of basophile material give a stippled appearance to the cytoplasm.

There is no sharp separation of trabecular hepatoma and adenohepatoma and a tumor may have alveoli representing both types. The transformation of the columnar type of basophile hyperplasia into trabecular hepatoma on the one hand and of tubular hyperplasia into adenohepatoma on the other is often evident. When tubules of liver cells are in continuity with adenomatous alveoli of the neoplasm the orientation of mitochondria and of linear basophile markings in relation to the base of the cell is maintained and is perhaps essential to the establishment of the adenomatous structure of the neoplasm.

Cholangiomas are derived from newly formed bile ducts which arise in great part at least from the immediate neighborhood of secondary portal spaces. Here there is apparently intense potential regenerative activity which results in the development of the precancerous lesion that has been designated cholangiofibrosis. The duct-like structures that are its essential constituent, like the parenchymatous cells at the site of basophile hyperplasia, have the ability to accumulate basophile material which has the characters of ribonucleic acid absorbing ultraviolet radiation and disappearing when treated with ribonuclease (10). Coincident with the accumulation of nucleic acid in the cytoplasm of these cells they enlarge, become columnar, and undergo active mitotic division. The formation of the adenomatous alveoli of the cholangioma

like those of the adenohepatoma is accompanied by the orientation of mitochondria and of linear basophile markings in a direction at right angles to the bases of the cells that form them and hence in the direction of their long axis. Otherwise the cytoplasm of the tumor cells has characters similar to those of the hepatoma and consists of mitochondria with basophile rims and fine basophile particles which give with appropriate stains a stippled appearance to the tumor cells.

CONCLUSIONS

Butter yellow (dimethylaminoazobenzene) causes degenerative changes in liver cells accompanied by chromatolysis of cytoplasmic structures that stain with basic dyes because they contain ribonucleic acid. These changes are profoundly modified by the protein content of the diet.

Chromatolysis is succeeded by focal regeneration with reaccumulation of ribonucleic acid in the cytoplasm of liver cells; these foci of basophile hyperplasia have their origin in the parenchyma surrounding portal spaces and consist of cells arranged in columns or as tubules with lumina.

Hepatomas arise from foci of basophile hyperplasia and corresponding with the arrangement of cells in these foci may be trabecular or adenomatous.

Butter yellow causes new formation of bile ducts which arise chiefly in the immediate neighborhood of secondary portal spaces and produce the precancerous lesion, designated cholangiofibrosis. These bile ducts may accumulate ribonucleic acid in their cytoplasm and undergo hyperplasia.

Cholangiomas arise from newly formed bile ducts that are the site of basophile hyperplasia.

Changes accompanying chromatolysis and basophile hyperplasia aid in the definition of structural elements of the cytoplasm and in the localization of ribonucleic acid with relation to the mitochondria.

BIBLIOGRAPHY

1. Opie, E. L., *J. Exp. Med.*, 1944, **80,** 219.
2. Opie, E. L., *J. Exp. Med.*, 1944, **80,** 231.
3. Heidenhain, R., *Arch. mikr. Anat.*, 1870, **6,** 368.
4. Garnier, C., *J. anat. et physiol.*, Paris, 1900, **36,** 22.
5. Held, H., *Arch. Anat. u. Physiol., Anat Abt.*, 1895, 396; 1897, 204.
6. Bensley, R. R., and Gersh, I., *Anat. Rec.*, 1933, **57,** 217, 369.
7. Pfuhl, W., Die Leber, in Handbuch der mikroskopischen Anatomie des Menschens, (W. von Möllendorf, editor), Berlin, Julius Springer, 1932, **5,** pt. 2, 235.
8. Caspersson, T., *Skand. Arch. Physiol.*, 1936, **73,** suppl. 8.
9. Brachet, J., *Compt. rend. Soc. biol.*, 1940, **83,** 88.
10. Opie, E. L., and Lavin, G. I., *J. Exp. Med.*, 1946, **84,** 107.

11. Roskin G., and Kharlova, G., *Compt. rend. Acad. sc. U. R. S. S.*, 1944, **44,** 389.
12. Caspersson, T., and Santesson, L., *Acta Radiologica*, 1942, suppl. 46.
13. Berg, W., *Anat. Anz.* 1912, **42,** 251; *Arch. ges. Physiol.*, 1922, **195,** 543.
14. Claude, A., *Science,* 1943, **97,** 451.
15. Claude, A., *J. Exp. Med.*, 1944, **80,** 19.

EXPLANATION OF PLATES

These photographs were made by Mr. Joseph B. Haulenbeek.

PLATE 4

FIG. 1. Normal liver showing diffuse basophile staining about portal spaces and basophile material in particles in cells about central veins. The liver was perfused with lanthanum acetate one-fifth molar, fixed in alcohol, formalin, and acetic acid, and stained by the Giemsa method. ×50.

FIG. 2. Chromatolysis after administration of butter yellow during 4 months with beginning basophile hyperplasia about portal spaces. Fixed in Zenker's fluid and stained by Giemsa. ×60.

FIG. 3. Focal hyperplasia in contact with a primary portal space after administration of butter yellow during 5 months showing two mitotic figures. Fixed in Zenker's fluid and stained by Giemsa. ×200.

FIG. 4. Focus of tubular basophile hyperplasia after 8 months of butter yellow administration in process of transformation into adenohepatoma. Fixed in Zenker's fluid and stained by Giemsa. ×95.

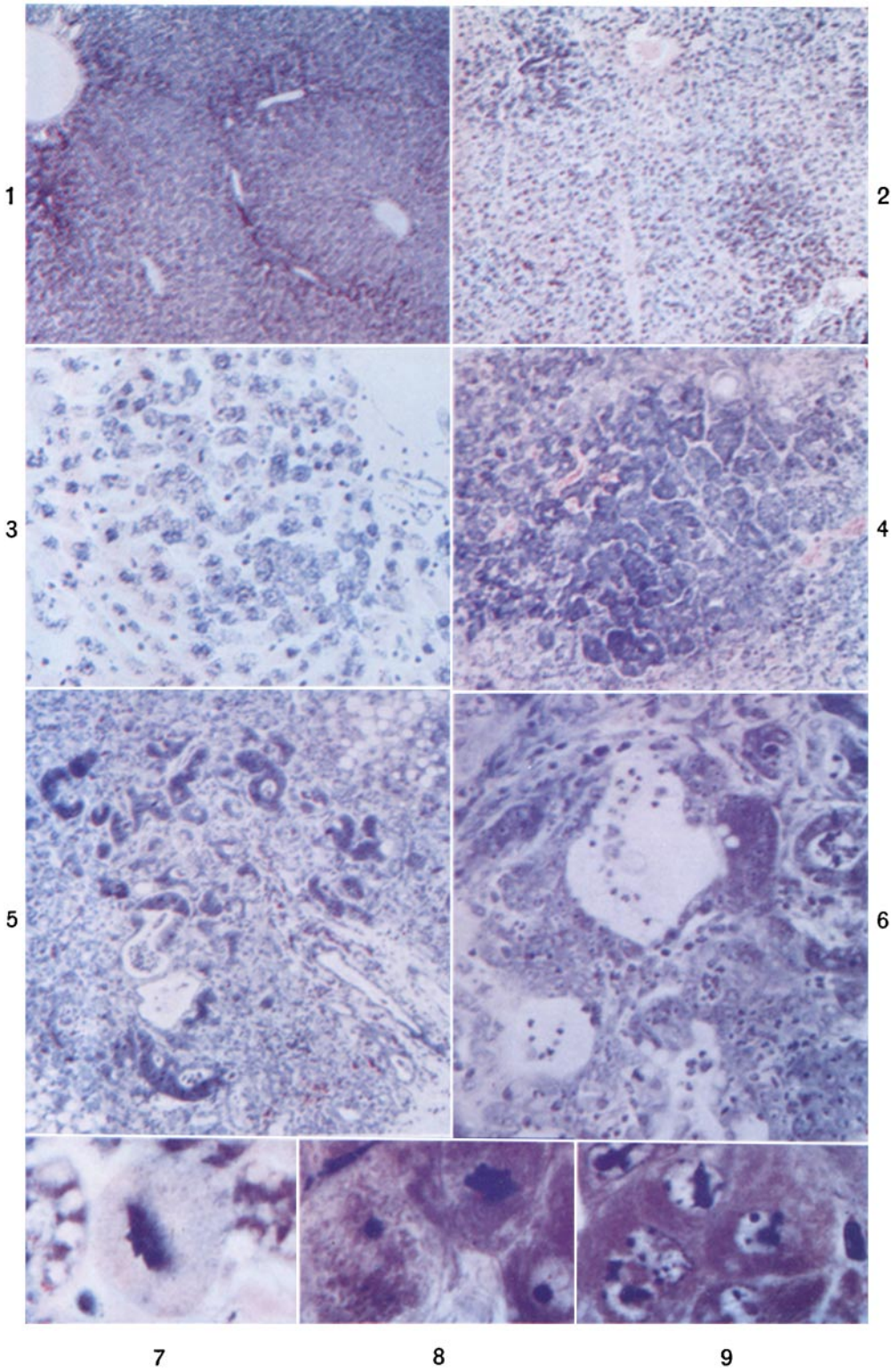
FIG. 5. Cholangiofibrosis about a secondary portal space after 6 months of butter yellow administration showing basophilia of cytoplasm of hyperplastic ducts. Fixed in Zenker's fluid and stained by Giemsa. ×115.

FIG. 6. Transformation of cholangiofibrosis with basophile hyperplasia into cholangioma after 6 months of butter yellow administration. Fixed in Zenker's fluid and stained by Giemsa. ×250.

FIG. 7. Mitotic figure in regenerating liver following partial extirpation, showing absence of basophile bodies and stippled appearance of cytoplasm. Zenker's and Giemsa. ×1000.

FIG. 8. Cell of hepatoma showing stippling of cytoplasm. Zenker; Giemsa. ×1000.

FIG. 9. Cell of hepatoma showing stippling of cytoplasm. Alcohol, formalin and acetic acid; Giemsa. ×1000.

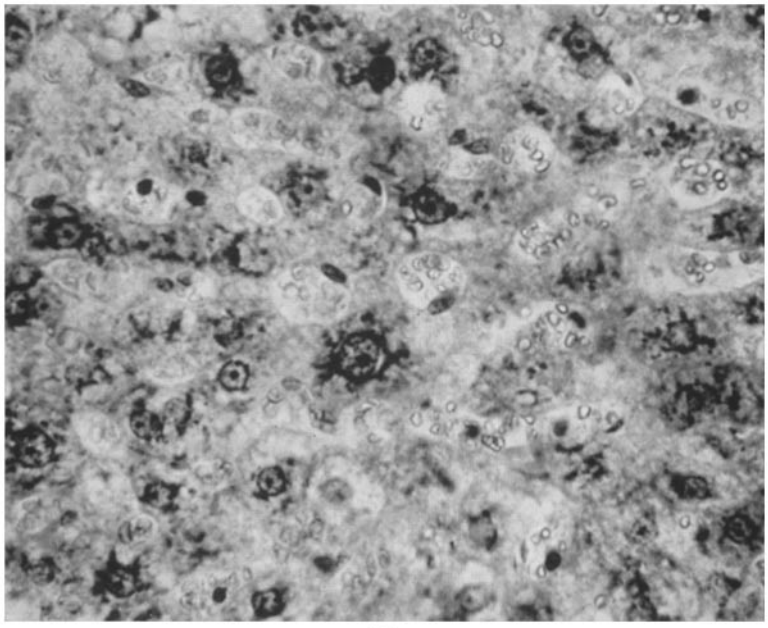


(Opie: Ribonucleic acid and hepatic tumors)

PLATE 5

FIG. 10. Chromatolysis after administration of butter yellow during 7 months. Showing clumping of basophile bodies about nuclei. Zenker; Giemsa. ×500.

FIG. 11. Chromatolysis after administration of butter yellow during 2 months. Showing disappearance of basophile bodies from central parts of cells. Zenker; Giemsa. ×500.



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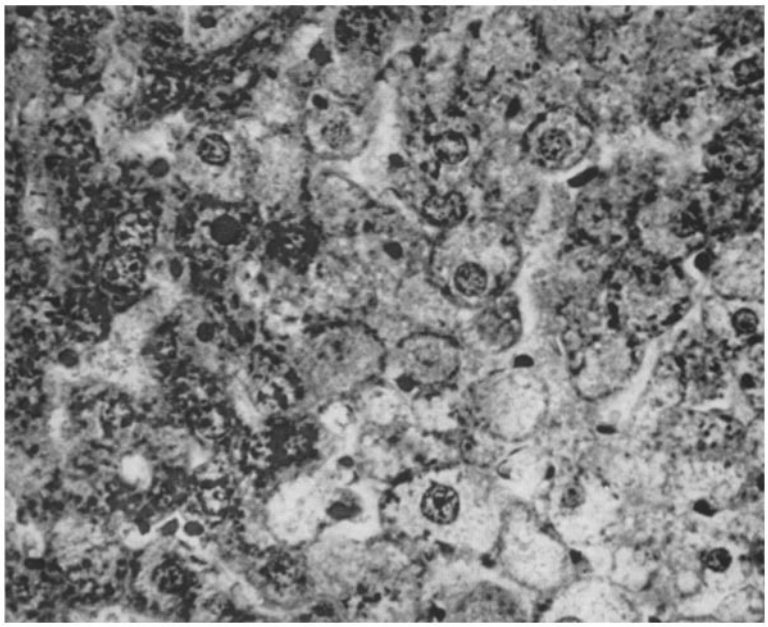

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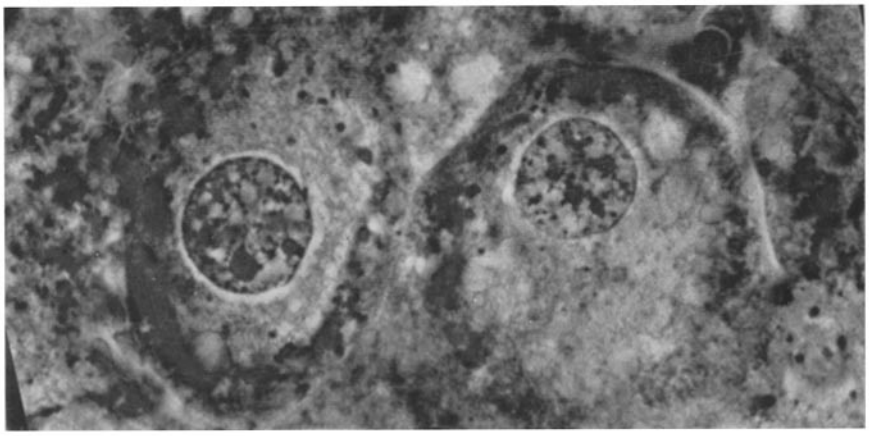
PLATE 6

FIG. 12. Higher magnification of liver cells with disappearance of basophile bodies from central parts after 6 months of butter yellow administration. The larger cell is vacuolated. Zenker; Giemsa. ×1000.

FIG. 13. Much enlarged hyperchromatic cell with basophile bodies surrounding the nucleus and acidophile mitochondria at the periphery of the cell, from No. 17, Table I, with both chromatolysis and focal hyperplasia after 7 months of butter yellow administration. Zenker; Giemsa. ×1000.

FIG. 14. Much enlarged cells from a focus of basophile hyperplasia from No. 22, Table I, with chromatolysis and focal hyperplasia after 8 months of butter yellow administration. The enlarged cells have mitochondria with basophile rims in the neighborhood of the nuclei and acidophile mitochondria at the periphery of the cell. Zenker; Giemsa. ×1000.



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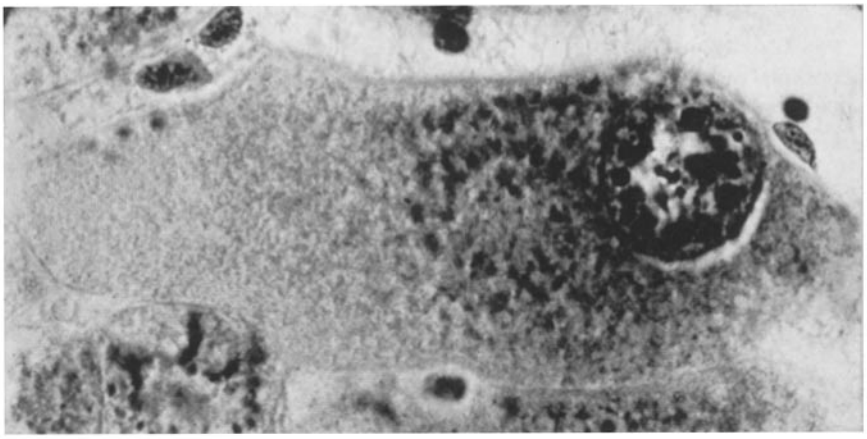

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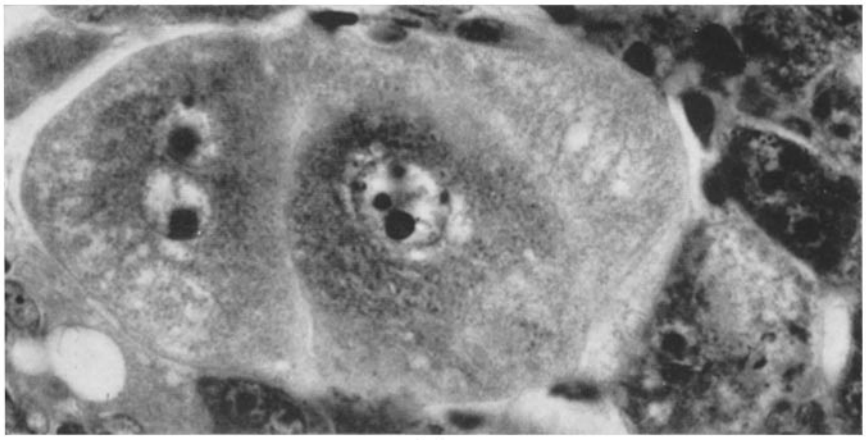

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(Opie: Ribonucleic acid and hepatic tumors)