GLYCOGEN INFILTRATION OF THE LIVER CELL NUCLEI *

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One of the most important functions of the liver is the synthesis of glycogen, a storage form of carbohydrate. This is a labile substance readily convertible into glucose, and although it is present in abundance in the cytoplasm of the liver cells after the ingestion of carbohydrates, it decreases or even disappears during fasting or starvation. On the other hand, glycogen is not found normally in the nuclei of the liver cells at any time. This report is devoted chiefly to a study of the pathological infiltration of liver-cell nuclei by glycogen, but during the course of this study it was possible to confirm certain earlier observations regarding the normal storage of glycogen in liver cells. These observations will first be reviewed briefly because the facts established by histological studies of glycogen deposition in the liver seem not to be very widely known.

Fixation and Staining

Glycogen is the only carbohydrate that can be studied histologically, being stained a brownish red colour by iodine and crimson by the more widely used Best's carmine stain. It is water-soluble and in order to retain it in the tissue for examination, water and predominantly aqueous solutions must be avoided before and after fixation. Ordinarily this end is achieved by fixing blocks of tissue in absolute alcohol and embedding them in celloidin.[1] However, in this study the following technic was employed:

Thin blocks of liver were fixed in a solution made up of 9 parts of absolute alcohol and 1 part of 40 per cent formaldehyde.† After fixation for 24 hours or longer the blocks of tissue were dehydrated in the usual manner, beginning with 95 per cent alcohol, and embedded in paraffin. Sections were cut at a thickness of 6 μ and mounted on ordinary glass slides. The paraffin was removed by xylol and the sections were then coated with a thin film of celloidin by pouring on a few drops of a 0.3 per cent solution of celloidin in equal parts of absolute alcohol and ether. Then the sections were run down to water following which the ordinary Best's carmine method of staining[1] was applied, the sections were freed from celloidin by acetone, dehydrated and mounted in Canada balsam.

By this method, thin sections can be readily obtained and handling of the sections is simplified by the fact that they are mounted on glass

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† Dr. Shields Warren, in a personal communication, stated that he has found Carnoy's fluid a more satisfactory fixative than either absolute alcohol or the formaldehyde and absolute alcohol solution used in this study.

slides at the outset. Altogether the procedure outlined is much quicker and easier than the usual celloidin method and gives equally reliable results.

Intracytoplasmic Glycogen

Best's carmine stain reveals the glycogen, if present, as small or large crimson granules distributed through the cytoplasm of the liver cells. Tissue culture studies,[2] however, have shown the glycogen to be actually in solution in the cytoplasm of the living cells and therefore its granular appearance in stained fixed tissue must be regarded as a constant fixation artefact. In livers containing large amounts of glycogen it is found to be fairly evenly distributed throughout the liver lobule, but as it is released from the liver it disappears first from the central cells of the lobule. Consequently, when present at all it is generally found to be most abundant in the periportal portions of the lobule. A tendency for the glycogen granules to be concentrated in the same side of each cell is due to the alcohol fixative having driven the glycogen ahead of it as it penetrated the tissues.[1]

In a large series of autopsy cases, in which the livers were stained for glycogen in the present study, a correlation could generally be shown between the amount of glycogen present and the elapsed time since food intake before death. In cases where death is unexpected and sudden, as from physical trauma or pulmonary embolism, glycogen is generally abundant in the liver. On the other hand, when there is a long agonal period of starvation, glycogen is generally scanty or absent.

The interval between death and the time of fixation of the liver up to 8 or 10 hours does not materially affect the appearance and quantity of glycogen in the liver, as estimated in sections stained with Best's carmine. Once the tissue is fixed in the alcohol-formaldehyde solution the glycogen apparently will remain unchanged for indefinite periods. Even after several years formaldehyde-fixed tissues will sometimes show the glycogen granules fairly well.

It is generally known [3] that the presence of large amounts of glycogen in the liver-cell cytoplasm will cause a characteristic appearance in ordinary paraffin sections stained with haematoxylin and eosin (Fig. 1). The cytoplasm, instead of presenting its more usual homogeneous character, becomes pale and fluffy in appearance. This appearance is sometimes mistaken for a degenerative change and it should be emphasized that it merely denotes the normal presence of glycogen which, of course, actually has been dissolved out during the fixation and staining process. Although this fluffy appearance is characteristic

and constant when large amounts of glycogen are present, lesser amounts of glycogen may cause scarcely any discernible change in the appearance of the cytoplasm in paraffin sections. Consequently, in order to exclude or detect the presence of small quantities of glycogen in the cytoplasm of liver cells, resort must be had to the Best's carmine stain. Thus the liver-cell cytoplasm may or may not contain glycogen under normal conditions.

Intranuclear Glycogen

The liver-cell nuclei normally do not contain glycogen. However, in 1883 Ehrlich [4] noted large glycogen-filled vacuoles in the liver cells in cases of diabetes mellitus. Later these vacuoles were shown to be the nuclei and since then it has been generally recognized that glycogen often infiltrates the liver-cell nuclei in diabetic persons. It has also been known for a long time, though not widely recognized, that glycogen frequently can be demonstrated in the liver-cell nuclei in non-diabetic persons.[5] For this reason Warren [5] believes that the mere presence of glycogen in the nuclei should not be regarded as a particularly significant lesion in diabetes. He does emphasize, however, that there is a significant reciprocal relation between the amounts of intranuclear and of intracytoplasmic glycogen. He also presents evidence to show that insulin promotes the deposit of glycogen in the liver-cell cytoplasm. In other words, he suggests that in the diabetic patient there tends to be an absence of glycogen-containing nuclei in the liver if he is well treated with insulin, while in uncontrolled cases glycogen-containing liver-cell nuclei are likely to be present in abundance.

In ordinary paraffin sections of the liver from cases of uncontrolled diabetes, one frequently sees peculiar vacuolated, empty-appearing nuclei in large numbers, situated in the peripheral cells of the liver lobules. It is usually assumed that these nuclei are the glycogen-containing ones. Similar vacuolated nuclei in similar distribution are also frequently seen in the liver in non-diabetic cases. In our earlier autopsy records this vacuolation of liver-cell nuclei has often been described as though representing some form of degenerative change, but almost never has there been any speculation as to the nature of the abnormality. It was the purpose of the study here reported to determine whether these vacuolated nuclei in the liver cells of diabetic patients are actually the ones which contain glycogen; whether this peculiar vacuolation of liver-cell nuclei is always associated with the intranuclear accumulation of glycogen and whether the presence of any considerable quantity of glycogen in the nucleus always produces

vacuolation visible in paraffin sections. In short, can this type of vacuolation of liver-cell nuclei be depended upon as a reliable indication of the presence of intranuclear glycogen?

The appearance, if not the nature, of these peculiar vacuolated liver-cell nuclei as seen in paraffin sections stained with haematoxylin and eosin is probably familiar to all who see routine autopsy material (Figs. 2 and 3). They are nearly always confined to the liver cells at the periphery of the lobules about the portal areas, although when present in large numbers they may approach the central part of the lobules. Thus, they occur predominantly in the cells which, during depletion of liver glycogen, are the last to give up their cytoplasmic stores of this carbohydrate. In their most striking form these vacuolated nuclei are very large, measuring up to 25 μ or more in diameter; they are generally somewhat irregular in outline, even crescentic or crenated. The nuclear chromatin and the nucleolus, if it is visible, are pushed to the periphery of the nucleus so that the nuclear membrane appears to have been overtraced with deep purple dye. The central part of the nucleus either appears completely empty and unstained, or it presents a pale, slightly opaque, violet or gray homogeneous appearance. The empty-appearing nuclei are indeed just that, for the diameter of the swollen nuclei being greater than the thickness of the histological section, the tops and bottoms of these nuclei have been cut off and one is in reality looking through an empty hole in the section. If histological sections of increasing thickness are prepared, the holes finally disappear in the thicker sections and only the nuclei with gray homogeneous centres remain. The latter, therefore, represent empty nuclei in which the nuclear membrane is intact over the upper or lower surface. By focusing up or down, this part of the nuclear wall is often clearly revealed.

In order to study the relation of this characteristic vacuolation of liver-cell nuclei to the intranuclear accumulation of glycogen, blocks of liver from 160 consecutive autopsy cases were fixed and stained for glycogen by the modification of the Best technic already described. Only newborn infants were excluded from this series, since preliminary studies had revealed no instance of vacuolation of the liver-cell nuclei in the newborn. The routine paraffin sections of the livers from the same 160 cases were examined, as well as the special sections stained for glycogen, but the examination of the two types of preparation was carried out independently and in each instance the specimen was graded as 0, 1, 2, 3 or 4 according to the examiner's estimate of the abundance of the peculiar vacuolated nuclei, described above, in the sections stained with haematoxylin and eosin, or the abundance of glycogen-containing nuclei in the sections stained with Best's carmine.

Without exception it was found that in those cases in which the peculiar vacuolated liver-cell nuclei were present in the routine sections, a roughly equivalent number of nuclei could be shown to contain glycogen in the liver sections stained with Best's carmine. The closeness of the numerical correspondence between the abnormal nuclei in the two types of preparation is indicated by the fact that in no instance was there a major disagreement between the grades which had been assigned to represent the estimated numbers of abnormal nuclei. Actually, the nuclei in question were more readily detected in sections stained with haematoxylin and eosin than in those stained for glycogen. That the empty-appearing nuclei seen in the former type of preparation really corresponded with those which contained glycogen in the sections stained with Best's carmine could scarcely be doubted after comparison of the sections under the microscope. Apart from the presence of one or more droplets of crimson-stained intranuclear glycogen in the sections stained to demonstrate its presence, the morphology of the abnormal nuclei was identical in the two types of histological preparation (Figs. 3 and 4). There was no instance of glycogen infiltration of liver-cell nuclei in which the peculiar nuclear vacuolation was lacking. Among the 160 cases studied, 94 were completely negative, that is, the liver-cell nuclei neither contained glycogen nor showed any sort of vacuolation. In 66 cases, or 41 per cent, small or large numbers of liver-cell nuclei were vacuolated and contained glycogen; in 24 of these cases, or 15 per cent of the total, considerable numbers of nuclei were found to be vacuolated by the presence of glycogen, and the abnormal nuclei were present in sufficient abundance that the sections had been assigned grades of 3 or 4.

The positive and negative evidence accumulated by the study of this first group of cases was supplemented by further evidence on the positive side obtained from the study of an additional group of 155 autopsy cases which were dealt with in the following manner. Blocks of liver tissue from each case were preserved in the alcohol-formaldehyde fixative in addition to the blocks of tissue taken routinely. The routine paraffin sections of liver from these same cases were studied and graded, as before, according to the numbers of abnormal vacuolated nuclei. In each case in which the peculiar vacuolated liver-cell nuclei were found in the routine sections, further histological sections were prepared from the specially fixed liver tissue and stained for glycogen. Again, in every instance, the peculiar empty-appearing nuclei seen in the routine liver sections stained with haematoxylin and eosin proved, in the sections stained for glycogen, to be glycogen-containing. In this group of 155 cases, 57 cases, or 37 per cent, showed the pres-

ence of vacuolated glycogen-containing liver-cell nuclei, and in 20 cases, or 13 per cent of the total, these abnormal nuclei were present in large numbers. This group of cases, combined with the first group studied, makes up a total of 315 cases. Vacuolated glycogen-containing liver-cell nuclei were demonstrated in 123 of these cases, or in 39 per cent, while in 44 cases, or in 14 per cent of the total, the abnormal nuclei were present in large numbers.

These findings, showing as they do a perfect correlation between glycogen infiltration and vacuolation of the liver-cell nuclei, were deemed sufficient to justify the conclusion that glycogen infiltration of the nuclei of the liver cells regularly produces a peculiar vacuolation of these nuclei which is easily recognizable in paraffin sections stained by routine methods. Moreover, these results indicate that this peculiar vacuolation does not occur except in association with infiltration of the nucleus by glycogen. It can therefore be stated that the characteristic vacuolation of liver-cell nuclei, already described, can be depended upon as a reliable indication of the presence of intranuclear glycogen.

Analysis of Cases of Intranuclear Glycogen Deposit

In the hope of determining the causes of glycogen infiltration of the liver-cell nuclei, the clinical and autopsy records were studied carefully in a series of cases in which large numbers of glycogen-containing nuclei were present in the liver. Only 44 such cases had been found among the 315 cases, as has been described in the preceding section of this report. However, the conclusions drawn from that study were made use of in the selection of additional cases of marked glycogen infiltration of the liver-cell nuclei. The routine sections, stained by haematoxylin and eosin, of the livers from 932 consecutive cases from the autopsy files, were examined with a view to selecting those cases in which the presence of many characteristically vacuolated liver-cell nuclei gave clear indication of glycogen infiltration of a degree equivalent to that found in the original 44 cases. This search yielded 96 additional cases, bringing the total to 140 cases available for analysis.

The hospital records of these 140 cases were examined. Among the features, other than the clinical diagnosis, of which particular note was taken were such factors as age, sex, total duration of illness, duration of severe illness, duration of critical illness, interval between the last intake of food and death, the use and quantity of intravenous glucose during the final illness, history of diabetes mellitus, the urinary findings, blood chemistry, blood pressures, blood counts, presence of fever, duration of fever, medication and symptoms such as dyspnoea and cyanosis. The autopsy protocols of these cases were likewise reviewed

and note taken of the principal anatomical findings, as well as other factors of possible importance such as state of nutrition and degree of arteriosclerosis. The histological sections of the liver in each case were reëxamined for lesions other than glycogen infiltration of the nuclei.

Clinical Features

It became apparent almost from the beginning of this analysis that many different kinds of cases were represented. Of the 140 cases, 32, or 23 per cent, were cases of diabetes mellitus. Since it is well established that glycogen infiltration of the liver-cell nuclei is common in diabetes, it was important to exclude the possibility of diabetes in the remaining supposedly non-diabetic cases. In deciding whether or not any given case was one of diabetes, the chief determining factor had to be whether or not such a diagnosis had been made clinically, but cases were considered as being cases of diabetes if there was any hint at all that they might have been. The figure of 23 per cent is therefore a maximum figure. On the other hand, in the cases considered to be non-diabetic, urinalysis showed the absence of sugar; blood sugar, when determined, was within normal limits, and nowhere in the case histories was the possibility of diabetes entertained. Perhaps if all the facts had been known, some of these cases might have proved to be cases of diabetes, but undoubtedly the great majority of the 108 cases considered to be non-diabetic were truly non-diabetic.

In only one case of diabetes was death attributed to uncomplicated diabetic coma, and for further consideration of the cases, diabetes, *per se,* was regarded simply as one of the many possible factors which might be responsible for glycogen infiltration of the liver-cell nuclei. The diabetic and non-diabetic cases were, therefore, considered together in the analysis of the cases for other factors of possible importance. This study did not reveal any other factor of constant causal significance nor any factor which was common to all cases, but the following features, briefly recorded, seemed noteworthy.

Among the 140 cases analyzed, the ages varied from 11 months to 82 years with an average of 53 years. It was apparent that the age of the patients was related to the disease from which they died and that there was no correlation between age and the presence or absence of glycogen in the liver-cell nuclei. Males constituted 54 per cent of the cases and females 46 per cent, a proportion between the sexes which does not differ greatly from that encountered within recent years among the cases coming to autopsy in this department.

No correlation could be established between the duration of illness and the presence of glycogen-containing nuclei in the liver. The short-

est interval between the onset of disease and death in a person previously in apparently good health was in a child, 2 years of age, who had died of diphtheria on the sixth day of illness. No correlation was observed with food intake nor with the intravenous administration of glucose. Likewise the medication employed in different cases presented no features common to all cases.

The blood pressure was moderately or extremely elevated in 40 per cent of the cases. There were no consistent findings on examination of the blood. However, the white blood cell count was elevated in many instances.

Associated Anatomical Lesions

The anatomical diagnoses recorded in the 140 cases covered a wide range, but it is noteworthy that there did not occur in the series a single case of uncomplicated traumatic death. This merely serves to emphasize the fact that glycogen infiltration of the liver-cell nuclei does not occur under normal conditions. The body was described as of average nutrition in 55 per cent of the cases, as obese in 25 per cent and as cachectic in 20 per cent.

Because of the variety of lesions, the cases could be grouped only in the most arbitrary manner according to the nature of the principal lesions which recurred most frequently. There was sometimes overlapping of certain features among cases placed in separate groups.

In 44 cases, or 31 per cent of the total, the outstanding feature at autopsy was the presence of marked inflammatory lesions usually accompanied by more or less extensive necrosis of tissue. Typical of such lesions were instances of multiple pulmonary abscesses, bilateral pyonephrosis, actinomycosis, gangrene of the lower extremities with septicaemia, phlegmon of the abdominal wall and extensive necrosis and secondary infection of bladder and vagina associated with carcinoma of the cervix. The locations of such lesions were most varied. Thirteen of the 32 cases of diabetes mellitus fell into this group.

In 70 per cent of the cases there was moderate or marked arteriosclerosis. There were 31 cases in which the principal diagnosis was some cardiac lesion leading to heart failure. In 14 of these cases there was coronary thrombosis and myocardial infarction. In 19 cases the principal lesion was a cerebrovascular one, either cerebral softening or haemorrhage. In combining these two latter groups, totaling 50 cases, or 36 per cent of the whole series, it is interesting to note that there were 5 cases in which there was a necrotizing arteriolosclerosis. Among these cases, too, necrosis of tissue was present in 33 instances in the form of myocardial infarction or encephalomalacia. Fourteen of the 32 cases of diabetes fell into this combined group of 50 cases.

There were 25 cases in which the outstanding lesion was a neoplasm. Among these were carcinomata of the breast, tongue, thyroid, larynx, bronchus, stomach, gallbladder, rectum and cervix. There was one lymphosarcoma, a chromaffin tumour of the adrenal, 2 cases of chromophobe adenoma of the pituitary, a suprasellar craniopharyngioma, a colloid cyst of the third ventricle and a glioblastoma. In many instances, extensive tumour metastases and necrosis with secondary infection were associated findings.

There were three cases of tuberculous meningitis, in two of which there was also a generalized miliary tuberculosis. All of these showed clinically a marked increase in intracranial pressure, and large tuberculomata of the brain were demonstrated in each case at autopsy.

One small but interesting group was made up of 4 cases of diphtheria in young children. In each, the diagnosis of diphtheria was established both clinically and pathologically. It is interesting to note that in 1900, Councilman, Mallory and Pearce,[6] in an extensive study of the pathology of diphtheria, described a peculiar vacuolation of the liver-cell nuclei as a frequent but inconstant finding. They regarded the lesion as a rare form of degeneration of the affected nuclei, but they realized that it was not peculiar to diphtheria for they noted that many such nuclei had been observed in a case of leukemia. Examination of the sections of liver from other cases of diphtheria in the autopsy files of this department revealed glycogen-containing nuclei in large numbers in at least one-half of the cases, but they were not a constant finding.

The remaining 14 cases could not easily be grouped or else were single examples of disease entities. There was 1 case of myasthenia gravis and 1 in which the patient died of pulmonary embolism 14 days after pregnancy had been terminated because of extreme toxemia.

A regrouping of some of the cases was prompted by consideration of the four cases of diphtheria, all of which were brought to hospital late in the illness and showed clinically symptoms and signs of suffocation. In six other cases as well, cyanosis and signs of strangulation were outstanding clinical features. One of these was a case of carcinoma of the tongue in which death finally occurred from respiratory obstruction. The same mode of death was observed in a case of carcinoma of the larynx, in another in which a large collar-like carcinoma of the thyroid compressed the trachea, and again in a case of extensive tumour infiltration of the mediastinum from a primary carcinoma of the breast. Another similar case was that of a patient with uncontrollable asthma who finally died with extreme respiratory embarrassment. The sixth case of this type was one in which the patient, a man 69 years old, dying of pneumonia, was found at autopsy to have an extremely nar-

row, sabre-shaped, calcified trachea. Dyspnoea and cyanosis were prominent in many of the cardiovascular cases also and in other cases with extensive pulmonary lesions such as abscesses, bronchial carcinoma or tuberculous cavitation. These could possibly be placed in the same group, but in them respiratory embarrassment was not so extreme nor could it be so readily evaluated from anatomical findings.

The four cases of tumour centering around the pituitary or floor of the third ventricle, as well as the one case of cerebral glioma and the three cases of tuberculous meningitis with markedly increased intracranial pressures, were thought possibly to form a significant group. However, examination of the histological sections of the liver in additional selected cases of this kind, in search of glycogen-containing nuclei, failed to reveal any constant correlation.

Apart from glycogen infiltration of the liver-cell nuclei, the liver itself in many cases showed no pathological alterations and in the remaining cases all manner of changes were observed. Chronic passive congestion of varying degree was a frequent occurrence, while other findings included fatty metamorphosis, periportal hepatitis and secondary carcinoma. Cirrhosis of the liver was present in only one case.

Thus, the analysis of 140 cases of marked glycogen infiltration of the liver-cell nuclei demonstrated only that this abnormality occurs as an accompaniment of a considerable variety of diseases. The more frequently associated conditions were diabetes mellitus; acute suppurative inflammatory processes; neoplasms; inflammatory, arteriosclerotic and neoplastic lesions of various kinds in which necrosis of tissue was a prominent feature, and lesions causing respiratory embarrassment with relative anoxia. The analysis provided no real clue as to the ultimate cause of glycogen infiltration of the liver-cell nuclei.

The grouping of the abnormal glycogen-containing nuclei in the periportal regions of the liver lobules suggests the possibility that some factor referable to the blood supply may be responsible for the occurrence of glycogen infiltration of the nuclei in these areas rather than elsewhere in the liver lobules. A significant relationship is also suggested by the fact that the periportal liver cells in which the abnormal nuclear accumulation of glycogen occurs are those which normally, during depletion of liver glycogen, are the last to give up their cytoplasmic glycogen stores.

Summary and Conclusions

Peculiar empty-appearing or vacuolated liver-cell nuclei with a peripheral arrangement of the chromatin are described as occurring not infrequently in paraffin sections of the liver from routine autopsy cases when stained by haematoxylin and eosin. By means of parallel glyco-

gen staining of sections of the liver, it is demonstrated that such abnormal, vacuolated nuclei invariably contain glycogen. Conversely, it is shown that glycogen infiltration of the liver-cell nuclei regularly produces the characteristic vacuolation of these nuclei which is easily recognizable in paraffin sections stained by routine methods. Such vacuolation of the liver-cell nuclei can, therefore, be depended upon as a reliable indication of the presence of intranuclear glycogen.

In a series of 315 unselected autopsy cases from which only newborn infants were excluded, glycogen infiltration of the liver-cell nuclei was demonstrated by glycogen staining in 123 cases, or in 39 per cent of the total. The abnormal glycogen-containing nuclei were present in large numbers in 44 cases, or in 14 per cent of the whole series.

In a series of 140 cases in which large numbers of liver-cell nuclei contained glycogen, the clinical data and autopsy findings were analyzed in the hope of determining the causes of glycogen infiltration of the liver-cell nuclei. Cases of diabetes mellitus made up 23 per cent of the series. The other cases, as well as the diabetic cases, showed a great variety of lesions. The analysis of clinical and autopsy data failed to reveal any factor of constant causal significance other than the presence of uncontrolled diabetes mellitus. Nevertheless, many of the features of the cases studied seemed worthy of note and these have been briefly recorded. The ultimate cause of glycogen infiltration of the liver-cell nuclei remains unknown.

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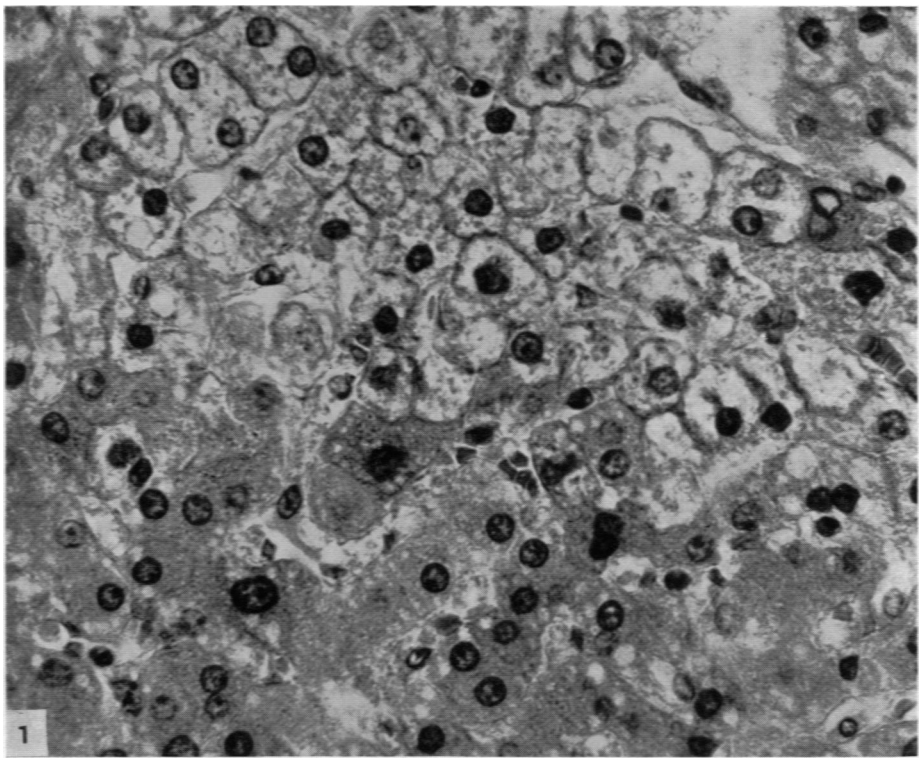
DESCRIPTION OF PLATES

PLATE 101

FIG. 1. Paraffin section of liver stained with haematoxylin and eosin, showing a patch of liver cells above in which the pale, fluffy cytoplasm is indicative of a rich glycogen content. These contrast with glycogen-poor cells below in which the cytoplasm has the more usual uniformly granular appearance. × 565.

FIG. 2. Paraffin section of liver stained with haematoxylin and eosin, showing large numbers of vacuolated, empty-appearing nuclei, particularly abundant near the periphery of the lobule. × 350.



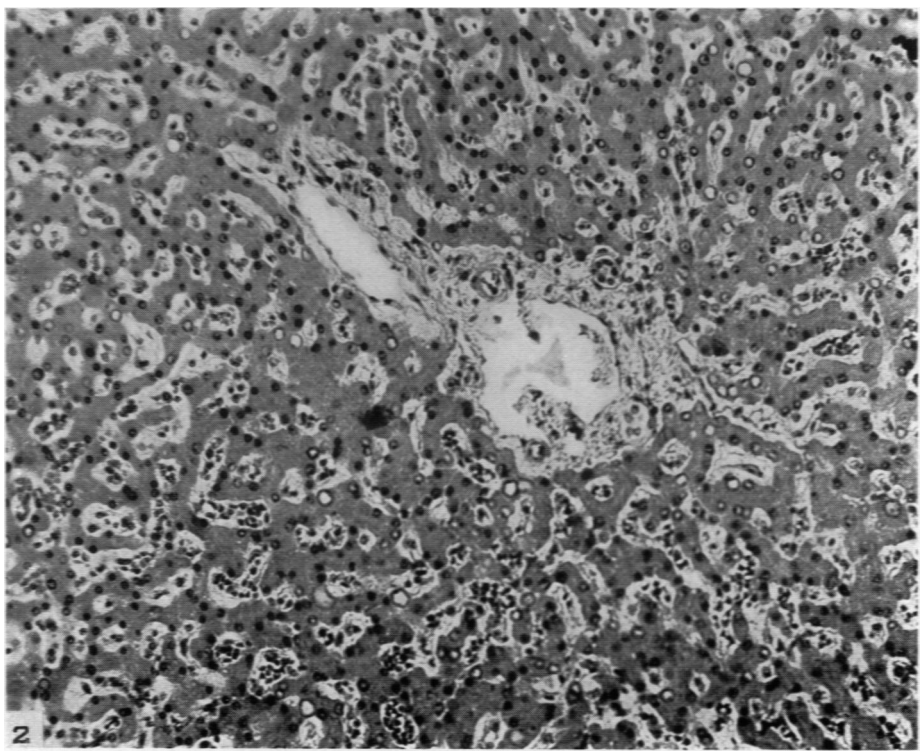


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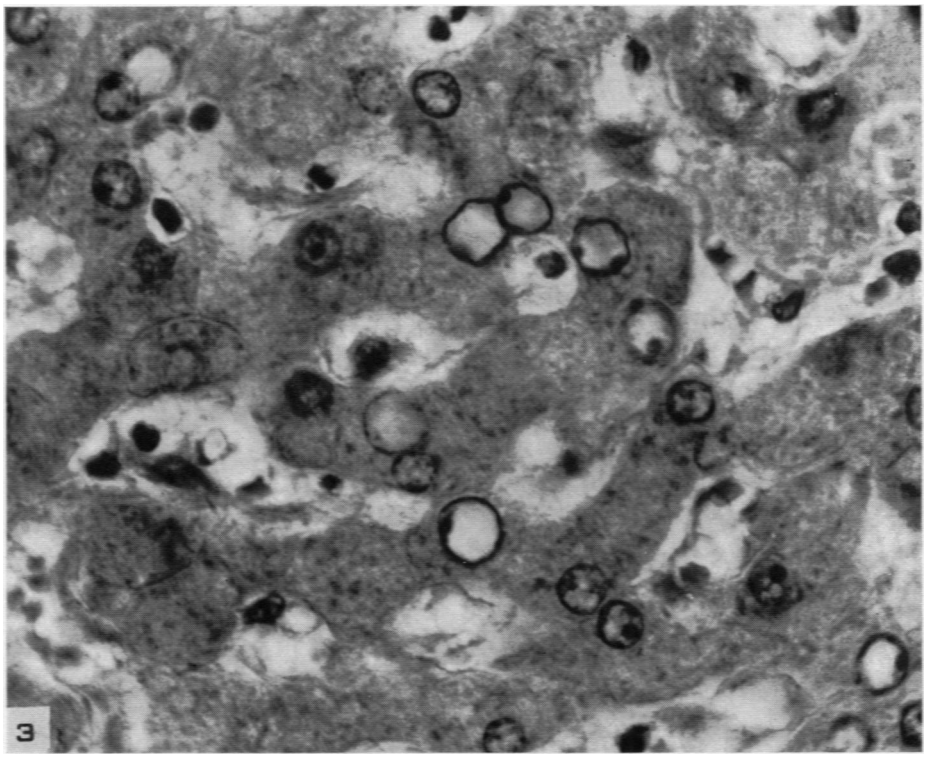
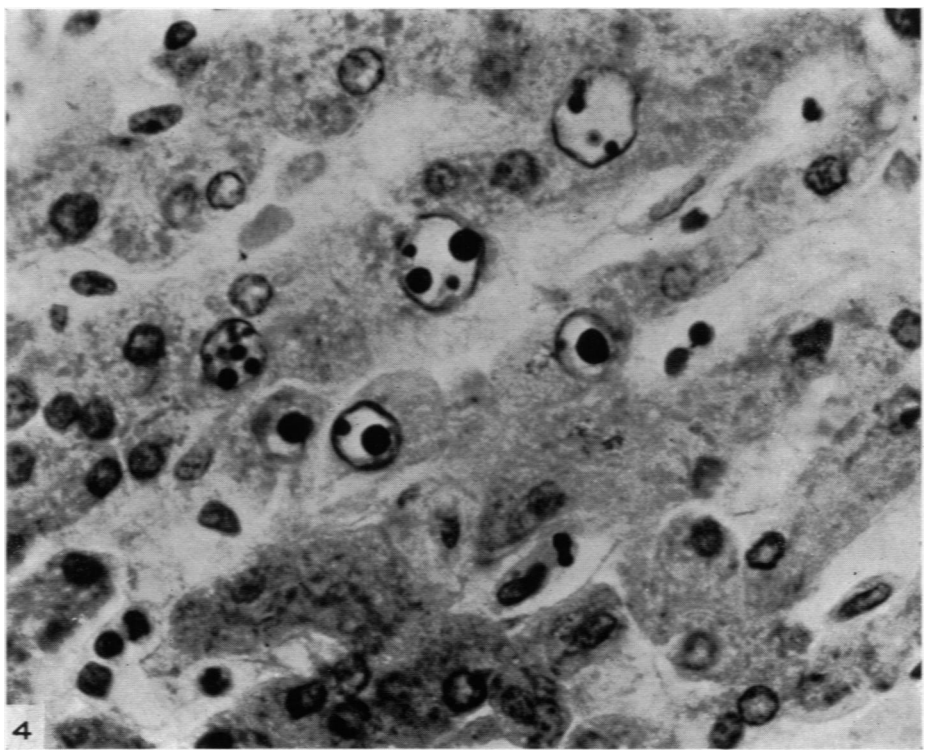
PLATE 102

FIG. 3. Paraffin section of liver stained with haematoxylin and eosin, showing the characteristic vacuolated liver-cell nuclei. Chromatin and nucleoli occupy an extreme peripheral position. × 855.

FIG. 4. Celloidin-treated, paraffin section of liver stained by Best's carmine method, showing glycogen infiltration of six of the liver-cell nuclei. The crimson-stained glycogen is represented in the photomicrograph by the black intranuclear masses. Apart from the presence of these droplets of glycogen, the appearance of these nuclei is identical with that of the vacuolated nuclei shown in Figure 3. × 855.





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