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# PATHOLOGICAL CHANGES IN PREGNANT MICE INFECTED WITH COXSACKIE B3 VIRUS AS A POSSIBLE CAUSE OF RETARDED FOETAL DEVELOPMENT

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**Summary.—Coxsackie B3 virus injected into mice on the eighth day of pregnancy resulted in foetal wastage and growth retardation. Although in apparent good health, the pregnant animals ate more food than the controls yet failed to increase in body weight as normal. This observation, together with the maternal autopsy findings of pancreatic acinar atrophy and hepatitis, suggests that the animals are subject to a manifestation of dietary deficiency attributable to an inability to break down and digest protein in their diet.**

**It would seem that whilst the possibility of the virus exerting a direct effect on the foetuses cannot be ignored, the action of the virus in reducing the state of health of the pregnant mother is largely responsible for the foetal effects seen.**

VIRAL infection in pregnancy has been established as an important cause of congenital malformation in children (Catalano and Sever, 1971; Davies, 1972; Tondury and Tondury, 1972). Often the pregnant mother exhibits fever, respiratory distress or other signs of illness. Although in these instances the foetal deformities may be partly due to the virus exerting a direct pathological effect on the foetal tissues, as in rubella (Cotlier *et al.*, 1968; Mims, 1968; Sever, 1970, 1971), it is likely that a reduction in the state of health of the mother is a contributory factor (Coid and Wardman, 1971, 1972; Edwards, 1972).

Currently, interest is being focused on the influence of mild viral infections in pregnancy and their possible relationship with intra-uterine growth retardation and abortions. In some of these infections the pregnant mother shows no overt signs of illness but in the offspring the features of infection are retarded intra-uterine growth (Coid and Ramsden, 1973), low birthweight (Davies, 1972) and a perinatal manifestation of heart disease, diabetes and mental retardation (Overall, 1972; Brown and Karunas, 1972).

Little information is available concerning the possible mode of action of sub-clinical viral infections in causing foetal anomalies of these types. It may be that a

net reduction in the foetal growth rate results from some subtle pathological changes elicited by the virus in the foetus or in the maternal tissues which adopt a particular function in pregnancy. In the case of infection with Coxsackie B viruses, either possibility is feasible in view of experimental evidence in mice which has shown that the pregnant animal is more susceptible to the cardiotropic and pancreatotropic action of the viruses (Dalldorf and Gifford, 1954), and that the viruses are able to pass readily across the placental barrier to infect the foetuses (Surjus, 1961; Suptel and Maximovich, 1964; Andrushchenko, 1968; Droughet and Levantis, 1968). At present, however, the aetiology of intra-uterine growth retardation in mice infected with Coxsackie B viruses is uncertain. The observation by Coid and Ramsden (1973) of a lower ratio of serum albumin to $\alpha_1$-fetoprotein in small foetuses from Coxsackie B3 infected mice may be evidence for a direct pathological effect of the virus on the foetal liver where these proteins are elaborated, or it may be the result of some other mechanism indirectly affecting maturation of the foetus.

In the present work some effects of Coxsackie B3 virus on the pregnant mouse are examined with a view to elucidating whether retarded foetal development resulting from infection at about mid-pregnancy may be attributed to a reduction in the state of maternal health which is not clinically apparent.

## MATERIALS AND METHODS

*Virus stock.*—The Coxsackie B3 virus used in these studies was from a stock strain maintained at the Public Health Laboratory Service at Colindale (London). It was derived from the original Nancy strain, passaged through suckling mice, isolated in primary rhesus monkey kidney cells and subsequently passaged 3 times through vervet monkey kidney (" vero ") cells. The undiluted virus suspension was shown to have a tissue culture infective dose ($TCID_{50}$) of $10^7$/ml.

*Animals.*—The mice used were of the " TO " outbred strain from the original Swiss stock (Theiler). Virgin females weighing between 25 and 35 g were used in each experiment. The females were placed overnight with proven males and the morning when vaginal plugs were found was designated Day 1 of pregnancy.

*Inoculation of virus.*—Groups of 20 pregnant mice were inoculated intramuscularly with 0·3 ml of undiluted virus suspension in tissue culture fluid on the eighth day of pregnancy. Control mice were given an equivalent dose of virus suspension that had been inactivated by heating to 56° for 30 min.

### *Experimental*

All animals were killed by cervical dislocation on the eighteenth day of gestation and were weighed with the foetuses *in utero*. Maternal livers were carefully dissected out and weighed. Thin slices of liver were fixed either in phosphate buffered formalin for routine histology or in formol calcium for histochemical demonstration of acid and alkaline phosphatase (Holt, 1969). Histological sections were stained using haematoxylin and eosin, the PAS technique, and oil red 0 for neutral fat. Acid and alkaline phosphatase were demonstrated using Burstone's naphthol AS-BI phosphate method with red-violet BL coupling agent (Burstone, 1962; Allison and Burstone, 1964). Other maternal tissues routinely fixed in buffered formalin for histological examination included pancreas, visceral fatty tissue, adrenals and kidneys. All sections were stained using haematoxylin and eosin, but in addition some sections of the pancreas were also stained using Gomori's (1941) aldehyde fuchsin method for $\beta$-cell granules.

The uteri were dissected from each animal and the foetuses and placentae examined and weighed.

In the course of the experiment, food consumption was monitored in test and control groups according to the following schedule: (a) pre-infection phase, 0–8 days of gestation; (b) infective phase, 9–14 days (according to the data presented by Droughet and Levantis (1968) virus is present in the maternal tissues at this stage); (c) post-infective phase, 15–18 days.

## RESULTS

The influence of Coxsackie B3 virus infection at about mid-pregnancy in TO mice is shown in Table I.

TABLE I.—*Effects of Coxsackie B3 Virus Infection in Pregnant Mice on Litter Size, Foetal and Placental Weights*

| Treatment | No. of animals | Average no. live foetus in each litter | Average no. resorption | Foetal weight (g) | Placental weight (g) |
|---|---|---|---|---|---|
| Live virus | 25 | 5·9 | 3·5 | 0·910±0·018 | 0·090±0·003 |
| Inactivated virus | 20 | 9·4 | 0·4 | 1·306±0·015 | 0·140±0·003 |

Results expressed as mean foetal and placental weights in g (not corrected for litter sizes) ± standard error.

The foetuses and placentae from pregnant mice injected with 0·3 ml of undiluted virus suspension on Day 8 of pregnancy were significantly smaller than those taken from animals injected with heat inactivated virus ($P = < 0{\cdot}002$), when examined after 18 days gestation. Exencephaly seen in a single foetus was the only incidence of gross deformity observed. The number of foetuses dying *in utero*, as indicated by the presence of sites of resorption, was greater in animals treated with live virus.

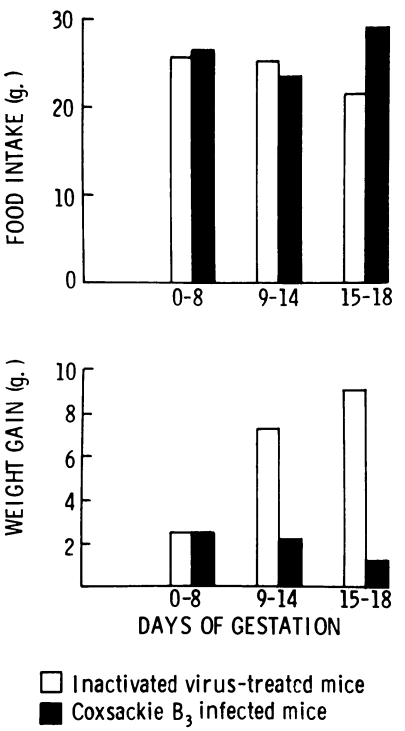



FIG. 1.—Food consumption and body weight gain in mice treated with live or inactivated Coxsackie B3 virus.

Food consumption and growth studies in pregnant mice infected with Coxsackie B3 virus show that in the period before infection food consumption and growth are similar to that of the control animals (Fig. 1). In the infective phase (9–14 days of gestation) food consumption fell slightly in the infected animals, but the increase in the body weight was significantly less. In the post-infective phase the food consumption of the infected animals was significantly greater (34%) than that of the controls, yet the increase in body weight was only about 11% of that seen in control animals. The net effect of infection at about mid-pregnancy was an increase in the total amount of food consumed but no corresponding increase in total body weight to the extent seen in control animals.

At autopsy, animals treated with an undiluted suspension of live Coxsackie B3 virus exhibited a marked proliferation of visceral fatty tissue, severe visceral adhesions and a noticeable reduction in the size of the liver and pancreas. In animals in which these changes were most marked, all or most of the conceptuses *in utero* were dead and in the process of resorption, the liver was pale and mottled in appearance and on occasions was only half the normal weight for the total body weight of the animal, and the pancreas was barely distinguishable from the surrounding fatty tissue.

*Liver.*—The liver weight relative to the body weight of infected and control animals is given in Table II. In pregnant animals injected with inactivated virus, the liver at 18 days gestation weighed 4·8% of the total body weight including the foetuses *in utero*. However, in the infected animals the relative liver weight was found to be significantly lower, being only 4·2% ($P = < 0{\cdot}001$).

TABLE II.—*Average Maternal Liver Weights Relative to Body Weights of Mice Infected with Coxsackie B3 Virus*

| Experimental group | Average body weight at autopsy (g) | Liver weight at autopsy (g) | $\frac{\text{Liver weight}}{\text{Body weight}} \times 100$ |
|---|---|---|---|
| Live Coxsackie B3 virus | 33·68 | 1·471 ± 0·068 | 4·21 ± 0·12 |
| Inac. Coxsackie B3 virus | 43·52 | 2·081 ± 0·062 | 4·80 ± 0·13 |

## EXPLANATION OF PLATES

FIG. 2.—Liver of pregnant mouse infected with live Coxsackie B3 virus showing marked vacuolation of periportal cells, dilatation and some nuclear pyknosis. H. and E. × 3·2 objective.

FIG. 3.—Cytoplasmic vacuolation and cell dilation in a periportal area of liver from an infected mouse. H. and E. × 20 objective.

FIG. 4.—Periportal fatty change in infected mouse liver. Oil Red O. × 5 objective.

FIG. 5.—Alkaline phosphatase activity in the pericanalicular areas of liver from a control mouse injected with inactivated Coxsackie B3 virus. Burstone's naphthol AS-BI phosphate method. × 8 objective.

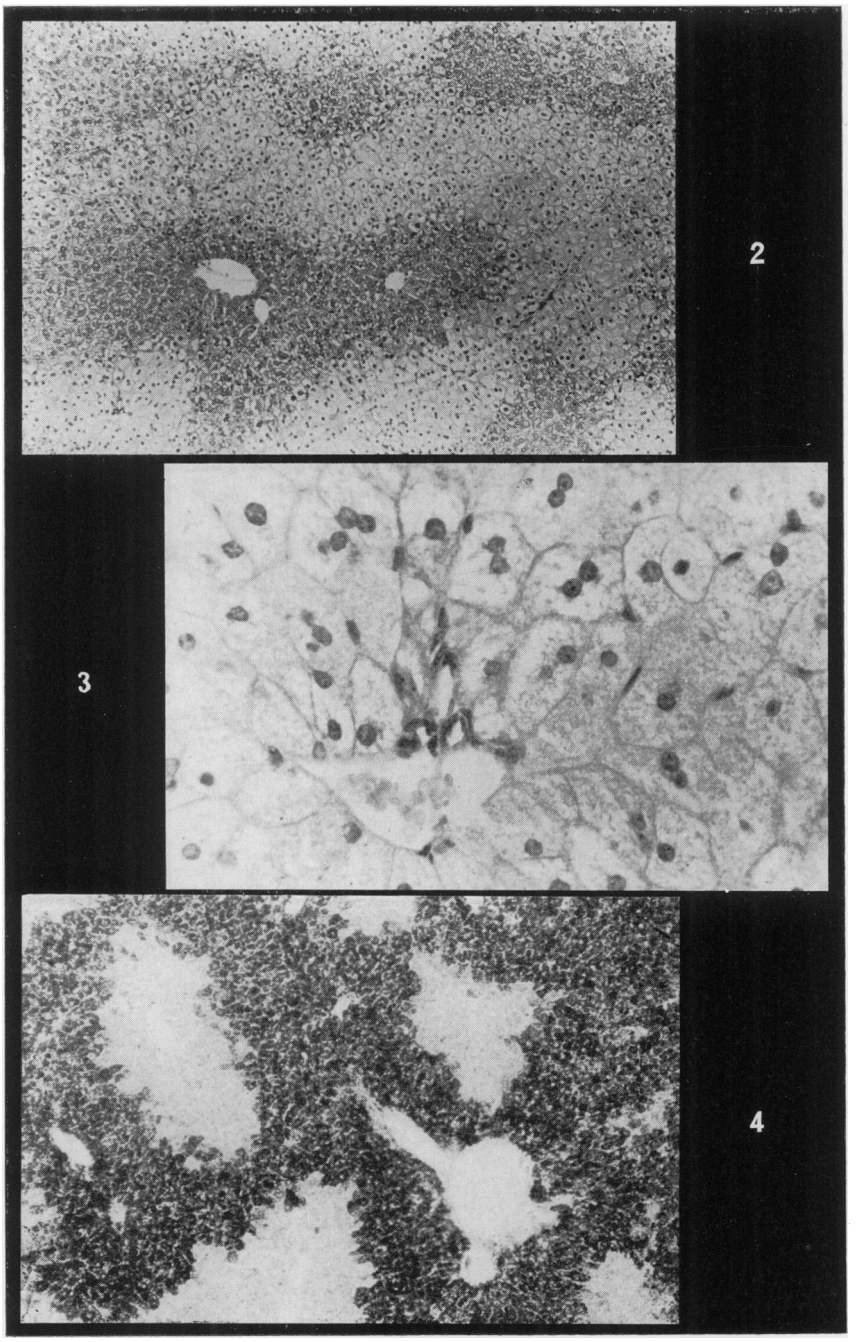
FIG. 6.—Alkaline phosphatase activity in the liver of a Coxsackie B3 virus infected mouse. Burstone's naphthol AS-BI phosphate method. × 8 objective.

FIG. 7.—Acid phosphatase activity in the periportal areas of Coxsackie B3 virus infected mouse liver. Burstone's naphthol AS-BI phosphate method. × 8 objective.

FIG. 8.—Acid phosphatase activity in the liver of a control mouse injected with inactivated Coxsackie B3 virus. Burstone's naphthol AS-BI phosphate method. × 8 objective.

FIG. 9.—Pancreatic acinar degeneration, loss of acinar structure and lymphocytic infiltration in a mouse infected with Coxsackie B3 virus. Note the lack of involvement of the tissues of the Islet of Langerhans. H. and E. × 8 objective.

FIG. 10.—Fatty tissue degeneration and necrosis in the ventral abdominal fat pads. H. and E. × 8 objective.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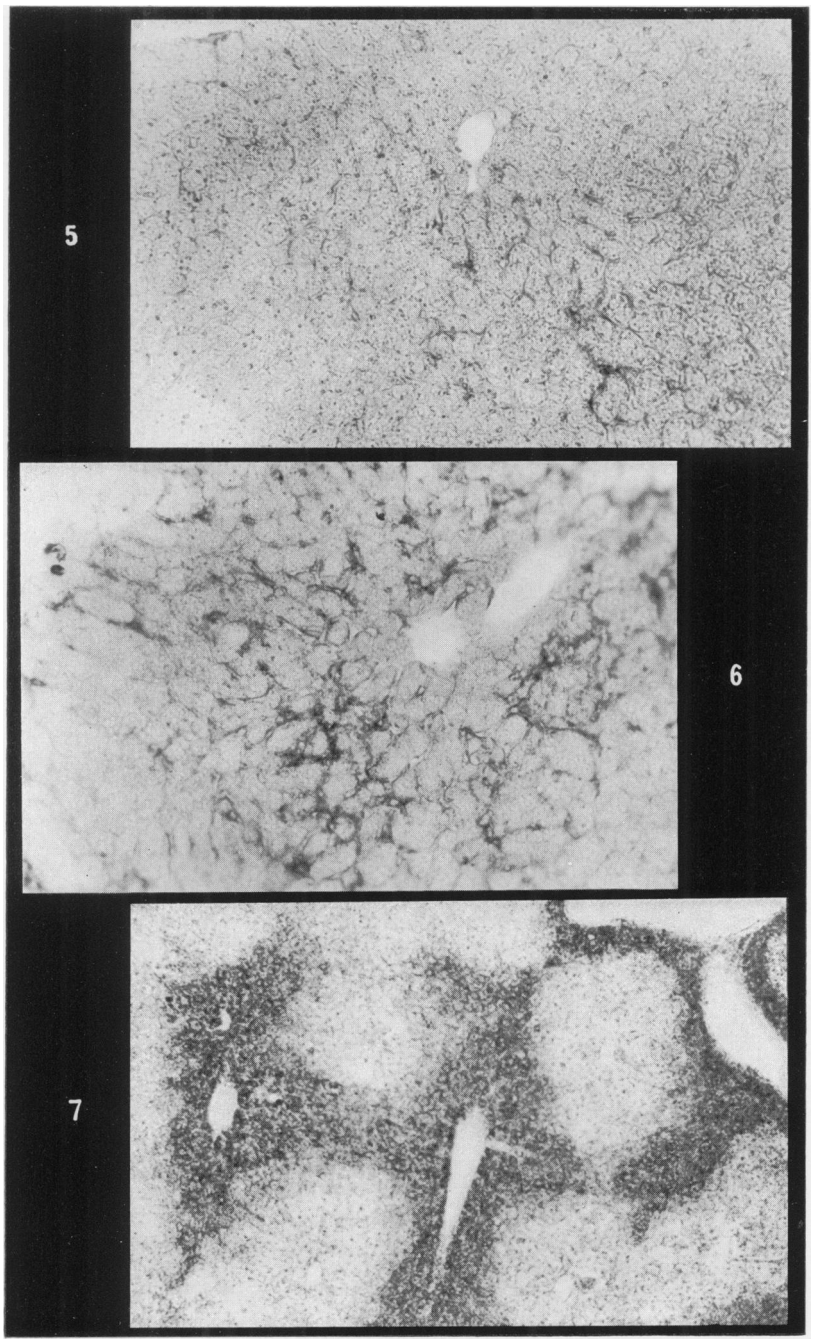
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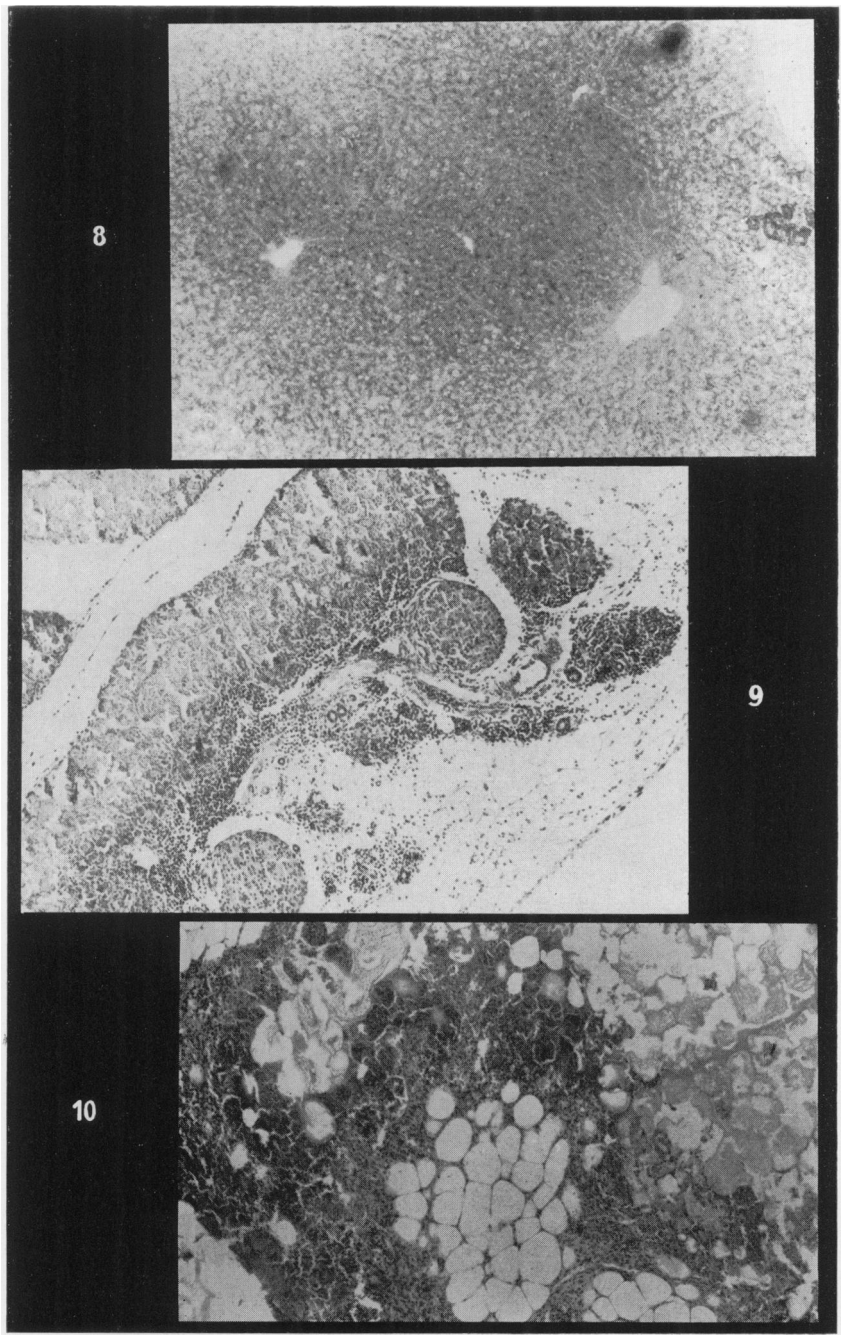


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Histologically, livers from infected mice exhibited a marked dilation and vacuolation of the cells in the periportal areas when examined after 18 days gestation (Fig. 2, 3). The nuclei of these cells were appreciably smaller than normal and were frequently pyknotic. In severely affected livers the periportal nuclei were situated centrally in the cells, with the cytoplasm being limited to fine strands. In the livers from most infected animals the cells in the centrilobular regions were rarely vacuolated and did not differ from normal. Small focal infiltrations of lymphocytic and mononuclear cells were occasionally seen in the centrilobular and periportal areas of livers from infected and control mice but in no instance was this condition pronounced or related to the treatment given.

Histochemically, the periportal areas of liver exhibiting profound vacuolation, dilation and nuclear pyknosis were associated with large deposits of neutral fat in the form of large globules and small droplets (Fig. 4). Although several of the control mice exhibited fatty livers, in these animals the fat was distributed throughout the liver lobule and was not restricted to the periportal regions as seen in the infected animals. Glycogen was present in the liver of both control and Coxsackie B3 virus treated animals and the distribution did not vary according to the treatment given.

Sections of livers from control animals stained for alkaline phosphatase activity showed that little or no enzyme was present other than in the walls of capillaries in the major portal tracts. A pale diffuse enzyme reaction product was present in the pericanalicular areas in some lobules but this was not pronounced (Fig. 5). In the livers of infected mice many of the canaliculi were clearly demarcated by an intense deposition of enzyme reaction product (Fig. 6). In these, the enzyme was only slightly more obvious in periportal regions than in other parts of the lobule.

Acid phosphatase activity, on the other hand, in infected mouse livers was clearly very much higher in periportal regions than in either midzonal or centrilobular areas (Fig. 7). High deposition of acid phosphatase reaction product in the form of large and small granules and diffuse staining was present in the periportal areas of most infected livers but elsewhere in the lobules enzyme activity was sparse. In the control animals acid phosphatase was appreciably less throughout the lobule and there was only a slight tendency for the enzyme to be less in the periportal areas (Fig. 8).

*Pancreas.*—In control mice the pancreas was yellowish in colouration, firm in consistency and readily distinguished from the surrounding fatty tissue with the naked eye. In infected animals where the fatty tissue was more abundant than usual, the pancreas was much diminished and was identified only as a fragile " web " of tissue extending from the vicinity of the spleen to the duodenum. Frequently it was not easy to distinguish the pancreas from the surrounding fat.

Histologically, pancreatic changes in the infected mice involved mainly the exocrine portions of the gland (Fig. 9) and included widespread acinar degeneration and necrosis, loss of zymogen granules and an infiltration of mononuclear and plasma cells. The cells of the pancreatic ducts appeared to be normal in morphology and distribution, and were not affected by viral infection.

In the Islets of Langerhans of infected mice, histological changes were rarely seen. Occasionally cells exhibited a slightly vacuolated cytoplasm with some nuclear pyknosis, but this condition was not marked in any animal examined. Although some reduction in the number of $\beta$-cell granules was present in a few sections from infected mice, this change was not thought to be consistent with the

treatment given. The other cell types in the islets were normal in infected animals.

*Visceral fatty tissue.*—Visceral fat was very much more abundant in infected mice, the two large posterior fat pads being particularly prominent. This fatty tissue, rather than being spongy in consistency as normal, was compact and exhibited small opalescent areas on the surface, suggestive of fatty tissue degeneration.

Histologically, the fat cells were degenerate and necrotic, the vacuoles containing basophilic material (Fig. 10). The tissue was widely infiltrated with polymorphonuclear leucocytes, plasma cells and lymphocytes. The fatty tissue necrosis was often associated with visceral adhesions and peritonitis.

*Kidney and adrenal gland.*—No pathological changes were identified in these organs in mice injected with undiluted live virus or inactivated suspension.

## DISCUSSION

Infection of mice at about mid-pregnancy with Coxsackie B3 virus resulted in an increase in the rate of foetal mortality, as indicated by the number of resorption sites, and a reduction in the weight of those young surviving to 18 days of gestation. In addition, severe pathological changes were evident in the maternal liver and pancreas.

The hepatic and pancreatic changes identified in the pregnant mice in this study are similar to those described in non-pregnant mice infected with Coxsackie B1 and B4 viruses and examined after 10 days (Burch, Tsui and Harb, 1972, 1973; Tsui, Burch and Harb, 1972; Harrison, Bauer and Murphy, 1972; Coleman, Gamble and Taylor, 1973). They have not been described, however, in pregnant animals infected with Coxsackie B viruses. Hepatitis was reported in a case of Coxsackie B3 infection in human pregnancy where the child was born mentally and physically retarded (O'Shaunessey and Buechner, 1962).

Despite the observation by Dalldorf and Gifford (1954) that in pregnancy mice become progressively more susceptible to the pancreatotropic action of Coxsackie B3 virus, in none of the studies so far carried out have pathological changes been noted in maternal tissues. In fact there seems to be a general opinion amongst workers that despite the infection the mother remains in overt good health throughout (Droughet and Levantis, 1968; Coid and Ramsden, 1973). In the present work the abnormal growth patterns, together with severe pathological changes in the liver, pancreas and visceral fat pads, indicate that the infected animals are not in good health and suggest that a reduction in the state of maternal health may contribute to abnormal foetal development.

The severe pancreatitis seen in the infected mice and the general loss of functional acinar units imply that these animals are incapable of breaking down and digesting complex dietary proteins, and are consequently subject to nutritional deficiency (Goldstein, 1968). This view is supported by the fact that the hepatic changes seen in the Coxsackie B3 infected mice are similar in type to those seen in animals fed experimental diets low in protein (Enwonwu and Sreenby, 1971). Changes characteristic of this condition include a reduction in the weight of the liver relative to the body weight of the animal, periportal vacuolation, fatty infiltration and a depression in the rate of protein synthesis.

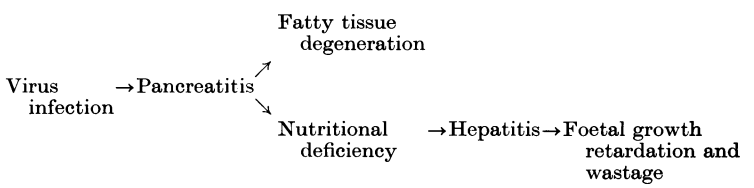
In our mice infected with Coxsackie B3 virus further evidence of changes in

cellular morphology was provided by observation of increases in the levels of acid and alkaline phosphatases. Previous observations of this kind in mouse livers subject to viral infection have been interpreted as changes in lysosomal activity and an alteration in cellular metabolism respectively (Allison and Burstone, 1964). Since lysosomes have been implicated variously in protein degradation, as occurs in tissue involution and morphogenetic remodelling of tissues (Ballard and Holt, 1968; Abraham, Morris and Hendy, 1969; Hayashi, Hiroi and Natori, 1973), it also seems likely that since high acid phosphatase activity occurs in areas exhibiting marked vacuolation, lysosomes may be involved in the breakdown and mobilization of labile protein in tissues subject to malnutrition (Swift and Hruban, 1964; De Duve and Wattiaux, 1966).

Dietary protein deprivation in pregnant animals leads to a production of small litters, longer periods of gestation and young which are smaller and less viable perinatally than normal (Zeman, 1967, 1969; Saxena and Roy, 1972; Enwonwu and Glover, 1973). According to Blaxter (1964), dietary intake in the rodent increases by as much as 30% in pregnancy and the liver increases in size due to hydration and an increase in protein and nucleic acid synthesis. This hepatic protein synthesis depends on the presence of at least one viable placenta *in utero* and an adequate supply of dietary nitrogen. In the event of dietary nitrogen deficiency, maternal protein reserves become depleted and foetal growth is retarded (Kalter and Warkany, 1959). If protein deprivation occurs at an early stage in pregnancy when the foetuses are in the process of differentiation, gross malformation may occur (Runner and Miller, 1956).

According to Munro (1964), protein deficiency may be caused experimentally by pancreatectomy or as a result of pancreatitis. These conditions lead to a gross reduction in the labile proteins of the liver in rodents and precipitate changes of a similar type to those seen in the Coxsackie B3 infected mice. A further change evoked by experimental pancreatitis is that of fatty tissue necrosis, which was also seen in the present work (Theve, 1973).

From the observations made in the present work it seems that infection in mice with Coxsackie B3 virus at about mid-pregnancy triggers off a sequence of events which lead to foetal wastage and growth retardation. Whilst the pattern of events suggested may be




the possibility cannot be discounted that the virus may elicit other changes in the pregnant animal, such as fever or systemic disturbances which may adversely affect the growth of the foetuses. Further work is indicated to examine in detail the sequence of changes which precede the pathological lesions seen in the mother at 18 days of gestation, and, in addition, to look for other effects of the virus on the pregnant animal. Whereas in the present work attention has been focused on the indirect effects of the virus on the growth of the foetuses, the possibility that the virus may bring about its effects on the foetus by a direct effect cannot be ignored. This aspect will form the subject of a future communication.

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## REFERENCES

ABRAHAM, R., MORRIS, M. & HENDY, R. J. (1969) Lysosomal Changes in Epithelial Cells of the Mouse Thymus after Hydrocortisone Treatment. *Histochemie*, **17**, 295.

ALLISON, A. C. & BURSTONE, M. S. (1964) Histochemical Demonstration of Changes in Liver Cell Enzymes following Infection with Mouse Hepatitis Virus. *Histochemie*, **3**, 462.

ANDRUSHCHENKO, N. I. (1968) The Effect of Coxsackie B2 Virus on Offspring of Albino Mice in Intra-uterine Infection. *Archs Anat. Histol. Embryol., Leningrad*, **54**, 92.

BALLARD, K. J. & HOLT, S. J. (1968) Cytological and Cytochemical Studies on Cell Death and Digestion in the Foetal Rat Foot: the Role of Macrophages and Hydrolytic Enzymes. *J. cell Sci.*, **3**, 245.

BLAXTER, K. L. (1964) Protein Metabolism and Requirements in Pregnancy and Lactation. In *Mammalian Protein Metabolism*. Ed. H. N. Munro and J. B. Allison. New York: Academic Press.

BROWN, G. C. & KARUNAS, R. S. (1972) Relationship of Congenital Anomalies and Maternal Infection with Selected Entero-viruses. *Am. J. Epidemiol.*, **95**, 207.

BURCH, G. E., TSUI, C. Y. & HARB, J. M. (1972) Pancreatic Islet Cell Damage in Mice Produced by Coxsackie B1 and Encephalomyocarditis Viruses. *Experientia*, **28**, 310.

BURCH, G. E.,TSUI, C. Y. & HARB, J. M. (1973) Hepatitis in Mice Infected with Coxsackie Virus B1. *Br. J. exp. Path.*, **54**, 249.

BURSTONE, M. S. (1962) In *Enzyme Histochemistry*. New York: Academic Press.

CATALANO, L. W. & SEVER, J. L. (1971) The Role of Viruses as Causes of Congenital Defects. *A. Rev. Microbiol.*, **50**, 255.

COID, C. R. & RAMSDEN, D. B. (1973) Retardation of Foetal Growth and Plasma Protein Development in Foetuses from Mice Injected with Coxsackie B3 Virus. *Nature, Lond.*, **241**, 460.

COID, C. R. & WARDMAN, G. (1971) Effect of Para-influenza Type 1 (Sendai) Virus Infection on Early Pregnancy in the Rat. *J. Reprod. Fert.*, **24**, 39.

COID, C. R. & WARDMAN, G. (1972) The Effect of Maternal Respiratory Disease induced by Para-influenza Type 1 (Sendai) Virus on Foetal Development and Neonatal Mortality in the Rat. *Med. Microbiol. Immunol.*, **157**, 181.

COLEMAN, T. J., GAMBLE, D. K. & TAYLOR, K. W. (1973) Diabetes in Mice after Coxsackie B4 Virus Infection. *Br. med. J.*, iii, 25.

COTLIER, E., FOX, J., BOHIGIAN, G., BEATTY, C. & DU PREE, A. (1968) Pathogenic Effects of Rubella Virus on Embryos and Newborn Rats. *Nature, Lond.*, **217**, 38.

DALLDORF, G. & GIFFORD, R. (1954) Susceptibility of Gravid Mice to Coxsackie Virus Infection. *J. exp. Med.*, **99**, 21.

DAVIES, P. A. (1972) Infection in Embryo-fetus and Newborn. *Br. J. hosp. Med.*, **13**, 36.

DROUGHET, V. & LEVANTIS, F. (1968) Le passage transplacentaire du virus Coxsackie B3 chez le souris gestante et la contamination foetale *in utero*. *Annls Inst. Pasteur, Paris*, **14**, 249.

DE DUVE, C. & WATTIAUX, R. (1966) Functions of Lysosomes. *A. Rev. Physiol.*, **28**, 435.

EDWARDS, M. J. (1972) Influenza, Hyperthermia and Congenital Malformation. *Lancet*, i, 320.

ENWONWU, C. O. & SREENBY, L. M. (1971) Studies of Hepatic Lesions of Experimental Protein–Caloric Malnutrition in Rats and Immediate Effects of Refeeding on Adequate Protein Diet. *J. Nutrit.*, **101**, 501.

ENWONWU, C. O. & GLOVER, V. (1973) Effect of Maternal Malnutrition during Pregnancy and Lactation on Hepatic Protein Metabolism in the Infant Rat: Biochemical and Ultrastructural Studies. *Am. J. clin. Nutrit.*, **26**, 3.

GOLDSTEIN, F. (1968) The Gall Bladder and Pancreas. In *Pathologic Physiology, Mechanism and Disease*. Ed. W. A. Sodeman and W. A. Sodeman. Philadelphia: W. B. Saunders Co.

GOMORI, G. (1941) Observations with Differential Stains on Human Islets of Langerhans. *Am. J. Path.*, **17**, 395.

HARRISON, A. K., BAUER, S. P. & MURPHY, F. A. (1972) Viral Pancreatitis: Ultrastructural Pathological Effects of Coxsackie B3 Infection in Newborn Mouse Pancreas. *Expl molec. Path.*, **17**, 206.

HAYASHI, M., HIROI, Y. & NATORI, Y. (1973) Effect of ATP on Protein Degradation in Rat Liver Lysosomes. *Nature, New Biol.*, **242**, 163.

HOLT, S. J. (1969) Factors Governing the Validity of Staining Methods for Enzymes and their Bearing upon the Gomori Acid Phosphatase Technique. *Exptl cell Res.*, (Suppl.), **7**, 1.

KALTER, H. & WARKANY, J. (1959) Experimental Production of Congenital Malformations in Mammals by Metabolic Procedures. *Physiol. Rev.*, **39**, 69.

MIMS, C. A. (1968) Pathogenesis and Viral Infection of the Fetus. *Prog. med. Virol.*, **10**, 194.

MUNRO, H. N. (1964) General Aspects of Regulation of Protein Metabolism by Diet and Hormones. In *Mammalian Protein Metabolism*. Ed. H. N. Munro and J. B. Allison. New York: Academic Press.

O'SHAUNESSEY, W. J. & BUECHNER, H. A. (1962) Hepatitis Associated with a Coxsackie B5 Virus Infection during Late Pregnancy. *J. Am. med. Ass.*, **179**, 169.

OVERALL, J. C. (1972) Intrauterine Virus Infections and Congenital Heart Disease. *Am. Heart J.*, **84**, 823.

RUNNER, M. N. & MILLER, J. R. (1956) The Congenital Deformity in the Mouse as a Consequence of Fasting. *Anat. Rec.*, **124**, 437.

SAXENA, U. C. & ROY, S. K. (1972) Effect of Transitory Protein Supplements on Early Fetal Development in Rats. *Ind. J. exp. Biol.*, **10**, 70.

SEVER, J. L. (1970) Viruses and the Fetus. *Int. J. Gynec. Obstet.*, **8**, 763.

SEVER, J. L. (1971) Virus Infections and Malformations. *Fedn Proc.*, **30**, 114.

SUPTEL, E. A. & MAXIMOVICH, N. A. (1964) The Possibility of Intra-uterine Infection with Coxsackie Virus in Mice Inoculated by Different Routes. *Acta Virol.*, **8**, 46.

SURJUS, A. (1961) Effets due virus Coxsackie B3 sur la souris gestante et sa transmission transplacentaire. *Annls Inst. Pasteur, Paris*, **100**, 825.

SWIFT, H. & HRUBAN, Z. (1964) Focal Degradation as a Biological Process. *Fedn Proc.*, **23**, 1026.

THEVE, N. O. (1973) Fat Necrosis; Studies in Rats with Experimentally Induced Pancreatitis. *Acta. chirurg. scand.*, Suppl. **434**.

TONDURY, G. & TONDURY, T. A. (1972) Course of Infection and Pathogenesis of the Human Embryo. *Revue suisse Zool.*, **79**, 179.

TSUI, C. Y., BURCH, G. E. & HARB, J. M. (1972) Pancreatitis in Mice Infected with Coxsackie Virus B1. *Archs Path.*, **93**, 379.

ZEMAN, F. J. (1967) Effect on the Young Rat of Maternal Protein Restriction. *J. Nutrit.*, **93**, 167.

ZEMAN, F. J. & STANBROUGH, E. C. (1969) Effect of Maternal Protein Deficiency on Cellular Development in the Fetal Rat. *J. Nutrit.*, **99**, 274.