the onset, the blood sugar returned to normal. Other possible causes such as encephalitis and head injury must be excluded. In Case I the history is unlike that of encephalitis, and the pyrexia can be accounted for by the bronchopneumonia. There was no evidence of head injury in Case II.

There remains only hypoglycaemia to account for the cerebral damage. Pathological changes, including chromatolysis, vacuolation of protoplasm, fragmentation of cell processes, pyknosis of nuclei, petechial haemorrhages, and oedema widely and irregularly disseminated through the nervous system, have been described by Terplan (1932), Baker and Lufkin (1937), and many others, in fatal cases of pancreatic tumour and insulin hypoglycaemic coma in man and animals. Baker and Lufkin, however, being unable to find similar changes in hypoglycaemic rabbits upon which necropsy was performed immediately after death, considered that most if not all the changes observed in their 3 human cases occurred post mortem. They believed, further, that similar changes could be found in normal brains at the same period after death and doubted whether hypoglycaemia had played any part in their causation. Baker (1939), however, later found incontrovertible evidence of hypoglycaemic cerebral damage. Among his 26 rabbits 7 were examined after a single induction of hypoglycaemia with convulsions. A great many petechial haemorrhages and widespread severe demyelination and encephalomalacia were demonstrated. In these experiments the hypoglycaemia, though doubtless severe, was limited to an hour's duration, and only one rabbit actually died in coma. Accornero (1939) and Ferraro and Jervis (1939) present corroborative evidence.

Tyler (1941) confirmed the presence of hypoglycaemic brain damage in cats even when convulsions were prevented by sodium pentobarbital (nembutal). He made the important observation that a fall of body temperature and the administration of drugs such as nembutal, which depressed cerebral metabolism, gave an appreciable measure of protection against brain damage. He attributed the damage to inadequate cerebral respiration, in which glucose plays a vital part. We cannot escape the conclusion that severe hypoglycaemia as a cause of cerebral damage and death is now well established. But hypoglycaemia pushed to the stage of convulsions is a common and comparatively safe method of treatment for schizophrenia. An interesting study of therapeutic hypoglycaemia in 25 cases of schizophrenia has been made by Day, Niver, and Greenberg (1938). After shock-doses of ordinary insulin, estimations of glucose in the blood and cerebrospinal fluid were made by Benedict's method. The Folin-Wu method, which I used, is sensitive to many non-glucose-reducing substances equivalent to 20 to 27 mg. of glucose per 100 c.cm. of blood; the Benedict method is insensitive to all but a few, equivalent to 4 to 8 mg. per 100 c.cm. of blood. It follows, therefore, that when comparing the results obtained by these different methods a correction of 15 to 20 mg. per 100 c.cm. of blood must first be applied. Out of 28 inductions of hypoglycaemia by Day and his colleagues, in one case the blood sugar remained for 2½ hours between 5 and 20 mg. per 100 c.cm. (Benedict). In every case consciousness was promptly restored on the administration of 15 g. of glucose intravenously, and no after-effects were observed.

The lowest blood sugar recorded in my two cases was lower than that present in any of the schizophrenic cases, allowing for the effect of the non-glucose-reducing substances. In fact, with the correction for the Folin-Wu method there was apparently a complete absence of glucose from the blood. This aglycaemia may have lasted from 1 p.m. till 3 to 4 p.m. (2 to 3 hours). In Case II the duration of aglycaemia was about 4 hours, and this implies that at some time during that period there was free insulin in the blood with no glucose upon which to act. In Case I also the same condition was almost certainly present. The effect of such a glucose vacuum may well be more destructive to the nervous tissues than any degree of mere hypoglycaemia in which the blood retains a reserve of glucose, however small. In view of Tyler's work previously mentioned it is probable that the destructive effect of aglycaemia was increased in both our cases by the application of radiant heat. It is possible that the tissues of diabetics, inured to a raised blood sugar, would be more susceptible than those of normal persons to damage from sudden extreme hypoglycaemia.

Conclusions

Too much must not be deduced from evidence based upon limited clinical material, but we venture to draw the following tentative conclusions:

1. Zinc-protamine-insulin, while constituting an advance in the treatment of diabetes and while diminishing the frequency and in most cases the severity of hypoglycaemic symptoms, adds seriously to the gravity of hypoglycaemic coma when established.

2. Patients who are exposed to shock and strain while under treatment with zinc-protamine-insulin may develop profound hypoglycaemia of rapid onset and fatal outcome.

3. Patients who develop hypoglycaemic coma while under treatment with zinc-protamine-insulin should be treated much more vigorously than is necessary for similar cases under ordinary insulin. We suggest the following measures in such cases:

(a) An immediate intravenous injection of 100 g. of glucose (400 c.cm. of 25% solution) by syringe or cannula—the same dose to be repeated within half an hour if necessary. In the absence of immediate intravenous facilities a still larger dose should be administered by stomach-tube.

(b) An immediate subcutaneous injection of adrenaline 15 minims and pituitrin 1 c.cm.

(c) Except perhaps in cases of extreme fall in body temperature no warmth should be applied and no cerebral stimulants given.

Summary

An account is given of two cases of insulin hypoglycaemic coma which failed to respond to prompt and moderately vigorous treatment. In each case there was a period of aglycaemia with probably excess of free insulin in the blood. The damaging effect of aglycaemia upon the brain and the relative safety of insulin-shock treatment for schizophrenia are discussed. Attention is drawn to the gravity of hypoglycaemic coma in diabetic patients under treatment with zinc-protamine-insulin, and measures which should prove effective are suggested.

My thanks are due to Drs. W. H. Jackson, R. Rogers, and H. C. Tuck, house-physicians, for constant vigilance and unremitting care in the treatment of the cases, and to Dr. W. Gilmour and his staff for biochemical and post-mortem examinations.

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A CASE OF HUMAN ISO-AGGLUTININ ANTI-M

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Antibodies for diagnosing the M-N blood-group factors have to be made by injecting appropriate red blood cells into an animal—nearly always a rabbit—and later absorbing suitably the rabbit serum to remove unwanted species antibodies. Because anti-M and anti-N do not occur naturally in man serum, and because M and N do not seem to be antigenic for man, the M-N groups can be neglected almost entirely in considering compatibility in blood transfusion. Wiener (1941) wrote: "Only 3 individuals have been observed to date with natural isoagglutinins anti-M, none with anti-N, among several hundreds of thousands

* Working on behalf of the Medical Research Council.

of individuals tested." He had previously (Wiener, 1939) mentioned three cases of anti-M and had given references to two of them. The first had been reported, without details being given, by Wolff and Jonnson (1933, p. 84), the second by Friedenreich (1937), and another case, which may be the third one referred to by Wiener, has been described by Moureau and Lambert (1939a, 1939b).

Details of the Case

Recently, as a result of testing, against the red cells of potential donors, the serum of a woman (M. W.) who might have needed a transfusion in connexion with parturition, an antibody, apparently anti-M, has been discovered. No transfusion was given. M. W. was 28 years of age and pregnant for the fourth time. Her blood group was ON and she was Rh-positive. Friedenreich's case was that of an infant aged 2½ months, of group ON, whilst Moureau and Lambert's case was that of a man of group A,BN aged 35. Friedenreich intended to find out what became of the anti-M as the child grew up, but it died of an epidemic disease when 9 months old.

Serum obtained from M. W. four months after her confinement was tested against the red cells of 20 persons belonging to group O. Equal volumes of serum and cell suspension—about 5% whole blood, as judged by eye—were mixed in 2 in. by 1/4 in. precipitin tubes; readings were made after the tubes had stood at room temperature for two hours. Undoubted and clear-cut agglutination was found in 14 of the mixtures; in some the reactions were seen by the naked eye, in others by using a microscope (see Taylor, Race, Prior, and Ikin, 1942). The 14 lots of cells which were agglutinated all contained the agglutininogen M; 5 were OM and 9 OMN. On the whole the reactions given by the homozygous M cells were stronger than those given by the heterozygous MN cells. The 6 donors whose cells did not react belonged to group ON, and so lacked the antigen M. When the reactions given by a known and an unknown antibody and the same panel of red cells are identical the odds in favour of the two antibodies being the same can easily be calculated. The probability that the apparent identity of the two antibodies is due entirely to chance is given by the formula $\frac{a! b!}{n!}$,* where n is the number in the panel

and a and b the numbers of positive and negative reactors. Applying the present figures, we get $\frac{14! 6!}{20!}$, or $\frac{1}{38,760}$, as the probability that the identity of the results is due merely to chance; in other words, the odds in favour of the antibody in M. W.'s serum being anti-M are 38,759 to 1. After absorption of the serum in the cold with OM cells the reaction, both in the cold and at room temperature, disappeared, while absorption with ON cells did not remove the antibody.

Table showing Titration of Serum of M. W.

Temperature	Serum Dilution	Cells			M. W.
		OM	OMN	ON	
2° C.	1/1	V	++	—	—
	1/2	V	w	—	—
	1/4	++	—	—	—
	1/8	—	—	—	—
Room temp.	1/1	V	(+)	—	—
	1/2	++	—	—	—
	1/4	—	—	—	—
37° C.	1/1	—	—	—	—

No reaction is scored as negative without a microscopical examination; reactions weaker than V were determined with a microscope.

C=Clumps of cells in clear surrounding fluid visible to naked eye.

V=Somewhat weaker reaction, with clumps distinctly visible to naked eye, although surrounding fluid is not clear.

++=Very big clumps under microscope: +=not quite such big clumps; (+)=less than ++.

w=Definite weak reaction in which there is a uniform distribution of very small clumps of about 6 to 8 cells.

Titration of the antibody in M. W.'s serum, detailed in the Table, showed that it was most active in the cold, less active at room temperature, and undetectable at 37° C.; the homozygous M reacted better than the heterozygous MN cells.

* We are indebted to R. A. Fisher for this formula. (6!= "factorial six" = 6×5×4×3×2×1=720.)

This iso-agglutinin anti-M, like those described by Friedenreich and by Moureau and Lambert, had some of the characteristics of a cold agglutinin.

Both Friedenreich, and Moureau and Lambert, examined the relatives of their patients, but found no evidence that the occurrence of the iso-agglutinin anti-M was familial. M. W.'s father is dead and her mother and one sister are OMN; a second sister belongs to group ON, but anti-M has not been discovered in her serum. The husband of M. W. is OM and all their children will therefore be OMN. It might be suggested that the anti-M in M. W.'s serum resulted from the stimulus provided by the antigen M, present in the cells of all her foetuses, but there is no evidence to support this possibility. Her four pregnancies were: 1935, female, 6 lb., alive and well; 1937, male, 6 lb., alive and well; 1939, miscarriage, 3/12; 1941, female, premature, alive and well. No child has shown any signs of erythroblastosis foetalis. The anti-M was not the result of a previous transfusion, for none had been given.

Summary and Conclusions

Iso-agglutinin anti-M has been found in the serum of a woman as the result of tests made to obtain a suitable blood transfusion donor for her.

Although the occurrence of iso-agglutinin anti-M must be exceedingly rare, the case here reported shows that its presence may interfere with the selection of a suitable blood transfusion donor for a person in whose serum this antibody appears.

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FURTHER DATA CONCERNING HUMAN FERTILITY

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Recent contributions to knowledge concerning the period of ovulation and successful fertilization in the human female (Weinstock, 1934) have thrown some doubt upon the validity of Knaus's theory of the relation between the dates of ovulation and onset of menstruation, and upon the deductions which may be made concerning the existence and duration of a pre-menstrual "safe period." Data quoted by Pryde (1941) and by Fleck, Snedeker, and Rock (1940), however, tend, within limits, to substantiate Knaus's views. Further data given below support the following hypotheses: (i) if fertilization does not occur menstruation follows approximately 15 days after ovulation; (ii) within limits there is a "safe" or infertile period a few days before the onset of the next menstrual cycle; (iii) an average "safe period" can be predicted with fair accuracy by a sufficiently extended study of the individual's menstrual periodicity.

The Present Investigation

The data concern all the acts of uncontraceptive intercourse during 31 menstrual cycles after which pregnancy did not occur and one cycle which was followed, as intended, by pregnancy. Pregnancy intended in cycles 30 and 31 was not fulfilled owing to the intermittent absence of one partner. There were no other acts in which contraceptive methods were used. An accurate record was kept of the dates and duration of each cycle, and the dates on which intercourse took place. A table was drawn up with vertical columns for the individual days of a cycle, starting with the first day of menstruation, data for successive cycles being recorded horizontally (see Chart). As each cycle was completed the last day was marked, and also