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INVESTIGATIONS IN A CASE OF MURDER BY INSULIN POISONING

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Since it was introduced in 1921 the potential dangers of insulin in the treatment of diabetic (Joslin, Root, White, and Marble, 1952) and psychiatric patients (Rees, 1949) and in diagnostic tests upon insulin-sensitive subjects such as those with hypopituitarism or Addison's disease have become well recognized. Deaths are known to have occurred in such patients as a result of accidental overdosage with insulin. Blotner (1954), in a comprehensive search of the literature, found eight recorded cases of attempted suicide by means of insulin and added one further case of his own; in three cases the outcome was fatal, and in all cases the subjects had first-hand knowledge of the effects of insulin. The present account describes the medical and scientific investigations in a case (*Regina v. Barlow*) in which a man was convicted of murdering his wife by injecting her with insulin. So far as we are aware, this is the first occasion on which such a charge has been substantiated and also the first in which insulin has been demonstrated in human tissue, other than the pancreas, after death.

Outline of the Case

On Friday, May 3, 1957, Mrs. E. B., a woman of 30 years, returned home from her work in a laundry for her midday meal. At about 11.20 p.m. on the same day she was found dead in the bath, which was empty when the police arrived.

The body, which was left undisturbed until seen by the pathologist at 3.30 a.m. on May 4, lay on its right side in the empty bath with the right leg drawn up so that the right knee rested against the side of the bath and the right foot lay beneath the left knee, which was slightly flexed. The arms were bent so that the hands lay in front of and slightly below the face; the fingers of the right hand were loosely curled round the left wrist and the left hand was closed. The position of the body as a whole was one associated with natural sleep. Blood-stained froth ran from the nose down the right side of the face on to the right hand. On retraction of the eyelids it was noted that the pupils were widely dilated, and the colour of the iris, which was only discernible as a faint line round the pupil, could not be determined. In the hair, which had settled on the side of the bath, were fragments of vomited food which were also found on the sides and bottom of the bath. Caught in the crook of the right elbow were 110 ml. of clean warm water. The skin was damp where it came in contact

with the surface of the bath, the bottom of which was also damp. The body was beginning to cool and rigor mortis was developing. The face was cyanosed and there was hypostasis on the right side of the body corresponding to the position in which it was lying. There were no obvious signs of violence, and the only other things in the bath were a wet face-cloth, a tablet of carbolic soap, and a soap-dish which lay together beneath the right loin and thigh.

The police, who searched the small semi-detached house in which the body was found, noted that the walls of the bathroom were dry and that there were no signs of splashing on the floor. In the hand-basin were two pillow-cases soaking in water and stained with vomit. In the main bedroom were some lady's sweat-soaked pyjamas, and a man's dressing-gown and pyjamas which were quite dry; one side of the double bed was disturbed as though someone had lain on it. In the kitchen downstairs were found a pair of vomit-stained sheets in a washtub, two hypodermic syringes, one of which was wet, four hypodermic needles, and many drugs, including quinine. No insulin or insulin phials were discovered.

The history, as obtained by the police from the husband, was that after her midday meal of fish and chips the deceased woman had done some housework and washing. At about 5 p.m. she had her tea, which consisted of toast, grapefruit, bread, butter, and tea with sugar, and then retired to bed at 6-6.30 p.m. At 7.30 p.m. she had asked to be called so that she could watch a television programme, but, in the event, she felt "too comfortable to move" and remained in bed; she had by this time changed into her pyjamas. At 8 p.m. her husband took her a drink of water; she was then "too tired" to say good night to her stepson. At 9.20 p.m. she called to her husband, who came upstairs and found that she had vomited on the bed. Having changed the bedclothes, he also retired to bed, whilst his wife, who complained that she was "feeling too warm," lay on the bed. Later she removed her pyjamas in the bedroom and went to have a bath whilst her husband remained in the bedroom reading. He dozed off to sleep, and when he woke at about 11.20 p.m. his wife had not returned. He found her submerged in the bath and apparently drowned, so he removed the plug to let the water out and then tried artificial respiration by "pressing on her abdomen." As he was unable to lift her from the bath and as his efforts at artificial respiration did not appear to have been successful, he then summoned assistance. The husband also said that his wife was pregnant.

The preliminary examination of the body described above suggested that the immediate cause of death had been

drowning. This was confirmed at the detailed post-mortem examination which was carried out at 5.45 a.m. on May 4 and is described in detail below. Specimens were taken from the body for chemical analysis, but no common poisons or abortifacients were found. A specimen of mixed heart blood, to which no preservative had been added, was shown seven hours later to contain glucose in a concentration of 210 mg./100 ml.

At this stage, three days after her death, it seemed difficult to explain why a healthy young woman should have drowned in her bath without any apparent signs of reaction to inhaled water; she lay as though asleep with no evidence of splashing or violence. It was concluded that immediately prior to her death she must have been unconscious and that the agent responsible for this had made her sweat and vomit and had produced the gross dilatation of the pupils. These observations suggested that the deceased woman may have been hypoglycaemic. The glucose concentration in the heart blood appears to contradict this hypothesis, but, as discussed later, such an observation has little value as an indication of the glucose level in the blood during life. The detailed post-mortem examination revealed no evidence of any disease which would suggest that the woman had become hypoglycaemic spontaneously, and the possibility that she had been injected with insulin was therefore considered.

On May 8 the body, which had lain on a mortuary slab at room temperature in mild weather conditions, was again examined. On this occasion and under better lighting conditions four hypodermic injection marks were found, two in each buttock just behind the greater trochanters. The tissues underlying these marks were therefore excised and it was shown, as described below, that they contained insulin in large amounts (84 units in 170 g. of tissue). The distribution of insulin was uneven, there being more in the left buttock, which was the site of the most recent injection mark, than in the right, and more in the superficial than in the deep tissues of the left buttock. The large amount found and the uneven distribution indicated that its presence was not the result of endogenous secretion. These findings, together with control experiments described briefly below, indicated that at the time of death the buttock tissues had contained at least 240 units of insulin. Such a dose must have been sufficient to produce either the soporific or comatose stages of the hypoglycaemic state (see Rees, 1949). No insulin was found in washings from the wet hypodermic syringe or needles.

On July 26 the husband of the deceased woman, a trained male nurse, was arrested and charged with the murder of his wife. He denied having injected insulin, but explained the presence of the injection marks by stating that on several occasions he had injected his wife with ergometrine maleate in therapeutic doses during the two weeks preceding her death in an attempt to procure abortion; he said he gave the last injection after the midday meal on the day of her death. The actions of this drug do not explain the signs and symptoms found in this case, and analysis of the urine and part of the buttock tissues did not reveal its presence.

The trial took place before Mr. Justice Diplock at Leeds Assizes in December, 1957, and the accused man was found guilty and sentenced to life imprisonment.

Post-mortem Examination

Two examinations were made; the first about six and a half hours after the body was found, and the second about four days later. On the first occasion artificial strip lighting with a bluish tinge was in use, and this was known to make identification of minute haemorrhages difficult. No such difficulty was encountered on the second occasion in bright daylight.

First Examination (5.45 a.m., May 4)

The body was that of a very well nourished young woman 5 ft. 3 in. (160 cm.) tall and proportionately built. The face

was cyanosed and the hypostasis was not fixed. The skin on the palms of the hands and soles of the feet was sodden, whilst elsewhere there were moles, freckles, and acne spots. Blood-stained froth issued from the nose, and petechial haemorrhages were found beneath the conjunctivae, the blood vessels of which were suffused with dark blood. No external evidence of violence was detected at this stage.

Internally the nose, mouth, and air passages contained blood-stained froth. The lungs were very bulky, congested, and wet; froth could be expressed from the smaller air passages, and subpleural petechial haemorrhages were found. The heart was apparently healthy, but was dilated on the right side, which was full of dark fluid blood. The uterus contained a normal foetus of about eight weeks' gestation with unruptured membranes; the cervix was healthy and a mucus plug was present. Petechial haemorrhages were found in the sheaths of the temporal muscles, and the meningeal vessels contained dark fluid blood. Routine examination of all other organs disclosed no abnormality other than that associated with acute venous congestion. No macroscopic evidence of disease was found in the pancreas, pituitary, thyroid, or suprarenal glands.

Histological examination of the lungs revealed oedema, small haemorrhages, and acute emphysema. The brain and spinal cord showed vascular engorgement and small haemorrhages. The heart muscle was normal. Vascular engorgement and some cloudy swelling were noted in the liver, spleen, and kidneys.

Bacteriological examination revealed no pathological organisms in the intestinal contents or the spleen.

It was concluded that death probably occurred between 5 p.m. on May 3 and 1 a.m. on May 4, the narrower limits being between 10 and 11 p.m. on May 3; that death was caused by asphyxia due to drowning; and that at the time of her death the deceased was probably under the influence of a drug.

Second Examination (9.15 a.m., May 8)

With the aid of a hand-lens four possible hypodermic injection marks were found, two lying close together on each buttock about 2 in. (5 cm.) behind the greater trochanters. One mark on the left side appeared to have a red track in the skin almost at right angles to the surface of the body and a small area of fat necrosis was found about 1-1½ in. (2.5-4 cm.) from the surface; these findings were confirmed microscopically. One of the marks on the right side also showed a doubtful vertical red track in the skin and, microscopically, a slight inflammatory reaction was found in the underlying fat. It was concluded that one mark in the left buttock was comparatively recent and had been produced a few hours before death, whilst one on the right buttock was slightly older.

A piece of skin, fat, and muscle (178 g.) including the hypodermic marks was excised from the right buttock; this is subsequently referred to as Sample R. A similar piece of tissue (131 g.) was cut from the left buttock (Sample L), but as this did not appear to include as much muscle as the sample from the right buttock a further piece (62 g.) of deep muscle (Sample DL) was excised. These pieces of tissue were stored without addition of any preservative in a refrigerator at about 4° C. for 15 days, after which they were examined, as described below, for their insulin contents.

Chemical Analysis

At 11 a.m. on May 4 a standard routine toxicological analysis was begun on specimens removed from the body at the first post-mortem examination. This analysis consisted of a search for several hundred poisons and drugs (including, *inter alia*, procaine, ergometrine, and quinine), and also certain biochemical investigations such as the measurement of the glucose content of the mixed heart blood and analysis of the urine for sugar and acetone. Specimens of vomit and of stomach and intestinal contents were examined separately, and 500-g. aliquots of liver and brain were analysed for the presence of poisons which might

have been injected; details of this routine analytical procedure will be published elsewhere (Curry, 1958a, 1958b). Nothing abnormal was found.

Traces of procaine penicillin were found in one of the hypodermic needles. In view of this, and because of the statement by the accused that ergometrine maleate had been injected into the deceased woman some hours before death, tests capable of detecting 1 μ g. of ergometrine or procaine in buttock tissue were applied to samples (approximately 60 g.) of the buttock tissue. No evidence of either alkaloid was found. Had she received a therapeutic dose of ergometrine seven hours before death, the alkaloid would have been detectable in as little as 5 ml. of the urine; in fact, none was found in 35 ml.

Tests capable of detecting the phenolic preservatives present in 0.2 ml. of commercial insulin solutions were also applied to the samples of buttock tissue. The results of these tests were not conclusive, but, since these substances rapidly disappear from the injection site, they suggest that the injections had been made some time before death occurred.

Because injections of insulin preparations containing added zinc do not appreciably increase the zinc content of tissues, analysis for zinc would not have been of any value in identifying the type of insulin used.

Blood-sugar Concentrations after Death

The glucose concentration in a sample of mixed heart blood taken from the body of the deceased woman about six hours after death and examined seven hours later, after storage without addition of preservative, was found to be 210 mg./100 ml. Urine taken at the same time contained neither glucose nor ketone bodies. The latter finding excluded the possibility that the woman was diabetic and in a hyperglycaemic coma at the time of her death, but the high blood-sugar level did not support the hypothesis that she had been hypoglycaemic.

It is known that the glucose concentration in the blood on the right side of the heart may rise for a period after death (Hamilton-Paterson and Johnson, 1940; Hill, 1941). This rise, often most marked in well-nourished subjects after asphyxial death, is due to glycogenolysis in the liver and subsequent diffusion of glucose into the inferior vena cava and the right heart. On the left side of the heart the glucose concentration usually falls progressively owing to glycolysis by the blood cells.

In 38 non-diabetic subjects, most of whom had died sudden or violent deaths, these observations were confirmed. The glucose concentration in blood drawn from the right side of the heart ranged from 20 to 671 mg./100 ml. and was frequently much greater than 210 mg./100 ml. That this rise was due to release of glucose from the liver after death was shown by the fact that it could be prevented if the inferior vena cava was ligated. The glucose level in blood from the left side of the heart was lower than that on the right side and ranged from 18 to 180 mg./100 ml.; if any rise occurred it was minimal (not more than 10 mg./100 ml.), and on one occasion only was a high level (440 mg./100 ml.) found.

In one case, that of a woman who committed suicide by drowning herself in a bath, a sample of mixed heart blood taken 29 hours after death contained glucose in a concentration of 355 mg./100 ml. The glucose levels in blood taken from the right heart (44 mg./100 ml.), inferior vena cava (56 mg./100 ml.), and left heart (50 mg./100 ml.) of another subject five hours after a slow death were low and approximately equal; 12 hours later the levels in the right heart (120 mg./100 ml.) and inferior vena cava (343 mg./100 ml.) had risen, while that in the left heart remained low (60 mg./100 ml.).

It is apparent that many factors, such as the glycogen content of the liver and coagulability of the blood, affect the degree and speed of the changes that occur in the glucose content of the blood after death. It is also apparent

that the high glucose content of mixed heart blood taken from the dead woman in the case described here does not exclude the possibility that she was hypoglycaemic before death.

Detection, Assay, and Identification of Insulin in Tissues

Although the structure of insulin, a protein of small molecular weight, is now known (Ryle, Sanger, Smith and Kitai, 1955), no chemical or physico-chemical method has yet been devised for its identification when mixed in small amounts with other tissue proteins. The only method available for identification and assay of insulin in the present case involved its extraction from the tissues in a crude form and examination of the biological activity of the extract. Chromatographic methods of separation were investigated and yielded some confirmatory evidence. A detailed account of the technical methods employed will be published elsewhere.

Extraction of Tissues

Crude extracts were prepared from the tissue samples by an improved method based on that originally described by Fisher and Scott (1934). By this method insulin is extracted irrespective of whether it is present as soluble insulin or in the form of one of the delayed-action preparations such as protamine zinc insulin or insulin zinc suspension. To assist in the identification of the insulin, portions of some of these extracts were further purified by a method similar to that described by Dodds and Dickens (1924); in this way a fourfold increase in insulin concentration was obtained.

After removal from the body four days after death the specimens of tissue were stored at 4° C. for 15 days, and subsequently at -20° C. until extracted. Extracts R, R₂, L, L₂, and DL were prepared from aliquots of the corresponding tissue samples.

In addition, many experiments were done on human tissue obtained at necropsy. Over 20 pieces of buttock tissue were removed from cadavers of both sexes, including a female drowned in a bath, and extracts prepared either immediately or after preliminary storage under conditions similar to those used for the tissues of the deceased woman; none of these extracts showed hypoglycaemic activity. Other pieces were injected with soluble insulin or protamine zinc insulin and then extracted immediately or after storage. It was shown that insulin could be recovered by the extraction method and that a considerable proportion of any insulin present in buttock tissue survives under the conditions of storage to which the specimens from the dead woman had been subjected. Although it is impossible to be precise, the control experiments suggested that about two-thirds of the insulin present in the injected tissue was recovered by extraction and that roughly half of the insulin originally injected survived the period of storage; these conclusions apply both to soluble insulin and to protamine zinc insulin. It was also shown that extracts of cadaver tissue injected with ergometrine exert no hypoglycaemic effect; nor does its presence modify the effect of insulin injected simultaneously.

The only control extracts mentioned in this account are extract E, prepared from buttock tissue removed from a fresh cadaver, and extract F, prepared from tissue (from the same cadaver) into which a known amount of soluble insulin had been injected.

Biological Activity of Extracts

Hypoglycaemic Action in Mice.—The mouse convulsion method of insulin assay described in the *British Pharmacopoeia* (1953) was employed; the results are shown in Table I. The activity in the extracts from 170 g. of tissue from both buttocks was equivalent to a total of 84 units of insulin. The uneven distribution in the two buttocks is clearly seen from Table I; widely differing concentrations were also found in two extracts (R and R₂) from

TABLE I.—*Mouse Assay of Tissue Extracts. Figures in Parentheses in the Last Two Columns are the Fiducial Limits of the Estimate (P=0.05)*

Tissue Sample	Weight of Sample (g.)	Portion of Sample Extracted (g.)	Extract Labelled	Weight of Extract (g.)	Insulin Content of Extract (Milli-units/mg.)	Total Insulin in Extract (Units)
Right buttock (R)	178	50	R	0.21	36 (31-41)	7.6 (6.6-8.7)
		10	R ₂	0.113	Between 0.5 and 1	About 0.1
Left buttock (L)	131	50	L	0.73	67 (56-79)	50 (41-58)
		10	L ₂	0.123	72 (60-86)	8.9 (7.5-10.6)
Left buttock (DL)	62	50	DL	1.04	16.5 (13.9-19.6)	17.2 (14.5-20.4)

different portions of the same tissue sample. The control extract E was inactive, while extract F showed hypoglycaemic activity consistent with the amount of insulin injected. It was shown that the convulsions observed in these experiments were due to hypoglycaemia; some of the

Hypoglycaemic effect of tissue extract and of insulin in guinea-pigs.

mice were sacrificed while convulsing and found to be hypoglycaemic, while others recovered from their convulsions when injected with glucose.

Hypoglycaemic Action in Guinea-pigs.—Two groups of fasted guinea-pigs were injected subcutaneously with extract L and with a comparable dose of soluble insulin respectively. The changes in blood-sugar concentrations which followed these injections were very similar (see Chart).

Action on the Isolated Rat Diaphragm.—With the method previously described in detail by Wright (1957), the glucose consumptions of hemidiaphragms incubated in the presence of the tissue extracts were compared with those of others incubated in buffer alone (basal uptake) or in buffer containing a known amount of insulin. The results of 13 experiments are shown in Table II, where it can be

TABLE II.—*Glucose Consumption of Rat Hemidiaphragms Incubated in Buffer Containing Added Insulin or Tissue Extracts*

Hypoglycaemic Agent Added to Incubation Medium*	Concentration of Agent	No. of Hemidiaphragms Used	Mean Glucose Uptake†	Insulin Effect‡
Nil	—	44	225 (43)	—
Insulin	1 m.u./ml.	27	416 (45)	191
Extract R	20 µg./ml.	12	419 (34)	194
“ L	40 µg./ml.	13	462 (43)	237
“ DL	20 µg./ml.	17	381 (31)	156

* The incubation medium was Krebs-Ringer bicarbonate buffer containing glucose.

† µg. of glucose/10 mg. dry weight muscle/90 minutes. Standard deviation in parentheses.

‡ Insulin effect = Mean uptake—mean basal uptake.

seen that all three extracts from the deceased woman's tissues have a marked stimulant effect upon glucose consumption; the same applies to extract F, but not to extract E (Table IV).

Identification of the Biologically Active Substance

The properties of the extracts were compared with those of insulin as follows:

Cysteine.—Mild reduction of insulin by means of excess cysteine at neutral pH destroys its biological activity by disrupting the S-S bonds in the molecule (du Vigneaud, Fitch Pekarek, and Lockwood, 1931). When the extracts were treated with cysteine, extract DL lost over 85% of its

hypoglycaemic activity in mice, and the stimulant effects of extracts R and DL on the rat diaphragm were also reduced (Table III).

TABLE III.—*Glucose Consumption of Rat Hemidiaphragm: Effects of Treatment of Extracts with Cysteine, Pepsin, and Anaerobic Conditions of Incubation.*

Hypoglycaemic Agent Added to Incubation Medium	Treatment Given	Insulin Effect*	
		Without Treatment	With Treatment
Insulin	Cysteine	254	86
Extract R		249	44
Insulin		192	61
Extract DL		174	57
Insulin	Pepsin	103	—17
Extract L		297	27
Insulin	Anaerobic incubation	194	—38
Extract R		204	—35

* Insulin effect = Mean uptake—mean basal uptake.

Proteolytic Enzymes.—Insulin can be destroyed by the action of pepsin (Butler, Dodds, Phillips, and Stephen, 1948), or by an extract of rat liver containing insulinase (Narahara, Tomizawa, Miller, and Williams, 1955). The hypoglycaemic effect of extract DL was almost abolished by treatment with insulinase, and pepsin destroyed the stimulant effect of extract L on the rat diaphragm (Table III).

Insulin Antiserum.—The serum of guinea-pigs sensitized by repeated injections of an oily suspension of insulin exerts a protective effect upon mice injected with an otherwise convulsive dose of insulin (Moloney and Coval, 1955). This effect is thought to be due to the presence of antibodies which are specific for insulin, although they are not species-specific. Serum from guinea-pigs sensitized with highly purified bovine insulin, when added to the incubation medium at the same time as insulin or as the extracts R, L, and DL, abolished the stimulant effects of these upon glucose consumption by the rat diaphragm (Table IV). Serum from normal control guinea-pigs had no such inhibitory effect upon insulin or upon the extracts L and DL.

TABLE IV.—*Effect of Serum From Insulin-sensitized and Normal Guinea-pigs on Glucose Consumption of Rat Hemidiaphragms Exposed to Insulin and Tissue Extracts*

Hypoglycaemic Agent Added to Incubation Medium	Concentration of Agent	Insulin Effect.*		
		Hypoglycaemic Agent Alone	Agent Plus Control Serum	Agent Plus Insulin—Antiserum
Insulin	1 m.u./ml.	268	350	18
“	1 “	317	—	86
Extract E	40 µg./ml.	—25	—	—
“ F	40 “	216	—	—14
“ R	20 “	145	—	—9
“ L	40 “	116	159	—17
“ DL	20 “	167	202	10

* Insulin effect = Mean uptake—mean basal uptake.

Anaerobic Incubation.—The normal stimulant effect of insulin on the rat diaphragm can be abolished if the incubation is carried out under anaerobic conditions (Ottoway, 1955). This was confirmed in the present experiments in the case both of insulin and of extract R when incubation was carried out in an atmosphere of nitrogen and CO₂ instead of oxygen and CO₂ (Table IV).

Comment

The results described above showed that the biologically active substance present in the extracts from the dead woman's tissues had the following properties: (1) It produced hypoglycaemia on injection into mice and guinea-pigs; (2) it stimulated glucose consumption by the isolated rat diaphragm; and (3) these biological effects were reduced or abolished by (a) cysteine, (b) proteolytic enzymes (pepsin and insulinase), (c) serum of insulin-sensitized guinea-pigs, and (d) anaerobic incubation.

These properties are characteristic of insulin, and no other known hypoglycaemic agent could have produced the same combination of effects. The effects of “synthalin,”

carbutamide, and tolbutamide were examined, but in one or other respect their actions differed from those of insulin and of the tissue extracts. Ergometrine was also excluded, since it has no hypoglycaemic effect. It was therefore concluded that the active material in the tissue extracts could only have been insulin, though it was impossible to decide what type of insulin preparation had been used.

Discussion

An account has been given of the methods adopted in the investigations and the results which led to the conclusion that the woman had been injected with a large dose of insulin prior to her death by drowning. The experience gained in these investigations enables us to make certain observations which might prove useful should a similar problem arise in the future.

The necessity for early attention to minute details in post-mortem examination is well emphasized by this case. The fact that the body was not disturbed after it was found resulted in the water being discovered in the crook of the elbow, and this significant finding threw considerable doubt on the accused man's claim that he had attempted artificial respiration. On the other hand, the injection marks on the buttocks were missed during the first examination because of the unsuitable quality of the mortuary lighting. The difficulty in assessing what material should be retained for histological examination requires careful consideration in cases of sudden death. In this case material from eight major organs was preserved, but the pancreas, pituitary, and suprarenal glands were not examined microscopically. Adequate samples, were, however, taken for the toxicological analysis, which followed a comprehensive routine. All common poisons and drugs were thus excluded at an early stage, and the absence of ergometrine in the viscera was established long before its use was alleged by the accused man.

The vomiting, sweating, and gross dilatation of the pupils which were observed in this case are not specific signs of hypoglycaemia. More objective evidence of hypoglycaemia would have been desirable, and in other cases might prove essential. Reference has already been made to the changes which occur in the glucose level of the blood after death. Both Hamilton-Paterson and Johnson (1940) and Naumann (1950) point out that glycolysis occurs more slowly in the cerebrospinal fluid after death, and that the glucose level in such a specimen taken after death gives a better indication of the blood-glucose concentration during life. It is, however, difficult to obtain a sample of cerebrospinal fluid uncontaminated with blood. Hamilton-Paterson and Johnson (1940) and Hill (1941) also suggest that in cases of suspected hypoglycaemia blood should be drawn from the left side of the heart within two hours of death; this is not always practicable. It therefore seems that no reliable method has been devised to establish the existence of hypoglycaemia before death. In all cases of sudden death the most useful information can be obtained by analysis of a specimen of peripheral blood from the femoral vessels preserved with fluoride; such a specimen cannot be used for blood grouping. The glucose content can be estimated and the result might be of some use in the assessment of the ante-mortem level; in addition it can be analysed for poisons and drugs, including alcohol. Somewhat surprisingly the subject of post-mortem blood-sugar levels is not referred to in many of the current standard textbooks on forensic medicine.

In the present case the possibility had to be considered that hypoglycaemia might have been due to natural causes. The fact that the deceased was a healthy young pregnant woman ruled out most of the possible causes of spontaneous hypoglycaemia, but there remained the possibility that an islet-cell tumour could have been responsible. Such tumours are often very small (Whipple, 1952) and might easily be missed during routine post-mortem examination. In this case the large amount of insulin recovered from the buttock tissues and its uneven distribution excluded the possibility

of endogenous secretion. Had the amount been much smaller the exclusion of an islet-cell tumour would have depended upon careful macroscopic and microscopical examination of the pancreas and upon proof that insulin was not present in tissues far removed from the sites of the injection marks.

The possibility that the woman's condition was due to ergometrine injections administered by the husband was excluded on several grounds. No ergometrine was found in the urine, the buttock tissues, or the viscera, and it was also shown that ergometrine does not exert effects in mice or on the rat diaphragm such as those produced by the tissue extracts from the deceased woman. In therapeutic doses it was shown that it had no effect upon the blood-sugar levels of normal obstetrical patients, that no perspiration or vomiting occurred, and that it had no consistent effect upon the pupils. In addition the clinical effects of ergometrine are known to be short-lived; its action starts in a matter of minutes and its effects pass off within an hour or two.

The successful extraction of insulin from tissues obtained from a body five days after death seems surprising, for it is generally assumed that insulin is a very unstable substance which is rapidly destroyed in the autolytic processes which occur in the body after death. Insulin is, however, stable in acid media, and lactic acid is released after death in peripheral muscular tissue. Proteolytic enzymes are released in large amounts into the viscera of the abdominal cavity, but autolysis in peripheral extra-abdominal tissues occurs only in the later stages of body decomposition. Insulin when injected into human buttock tissue cannot be recovered if the tissue is first allowed to putrefy, and for this reason it is essential to obtain tissue samples before putrefaction begins and to store them without addition of preservative at 0° C. until they are extracted. There is no available chemical or physico-chemical method for the detection and identification of insulin in crude protein extracts. Its biological activity has to be detected and then compared with that of the hormone itself. By means of the rat-diaphragm technique it is possible to detect less than 1 mliunit. The mouse-convulsion method is less sensitive, the minimum amount detectable being of the order of 10 milliunits, but, on the other hand, it is more satisfactory for quantitative assay purposes.

Summary

An account is given of a case in which a woman was found drowned in her bath. The post-mortem examination and the findings at the scene where the body was found suggested that prior to her death the woman was unconscious. The absence of common poisons in the tissues of the body and in the urine, the presence of vomited food on the bedclothes and in the bath, the sweat-soaked pyjamas, and the grossly dilated pupils suggested that the woman was hypoglycaemic. The subsequent finding of injection marks on her buttocks led to a search for insulin in the underlying tissues. A large amount of insulin was recovered (84 units), and this is thought to represent about a third of the amount present at the time of her death and an unknown lesser fraction of the amount which was injected. The woman's husband, a trained male nurse, was accused and convicted of his wife's murder. Some of the problems which arose during the medical and scientific investigations in the case are discussed.

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CASE FOR INDUCTION OF LABOUR IN TREATMENT OF HAEMOLYTIC DISEASE OF THE NEWBORN

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In a series of papers in the *British Medical Journal*, Walker and his colleagues have reported their experience in the management of cases of haemolytic disease of the newborn (Walker and Murray, 1954, 1956; Walker, Murray, and Russell, 1957; Walker and Neligan, 1955). Their analysis has been based largely on a series of cases referred for treatment in the Newcastle United Hospitals and in other centres in N.E. England during the past ten years. The management of the cases reported in those studies has been modified in the light of experience, so that the earlier reports refer to methods of treatment rather different from those used currently.

In the initial studies Walker and his colleagues subscribed to the view that the induction of labour had no place in the treatment of the infant suffering from haemolytic disease of the newborn. This view was based largely on the results of a clinical trial which suggested that prematurity greatly increased the risk of death in affected infants (Mollison and Walker, 1952; Armitage and Mollison, 1953). More recently, however, the Newcastle group has suggested that induction of labour should be used in cases where an Rh-immunized mother has already had a stillbirth due to

Rh incompatibility in a previous pregnancy. This suggestion has been incorporated in the second edition of *Blood Transfusion in Clinical Medicine* (Mollison, 1956). Subsequently, as a result of a study of the influence of the father's genotype on the severity of haemolytic disease in the affected infant, Murray (1957) has suggested that induction of labour might be justified also in cases where the father is shown to be R_2R_2 (cDE/cDE), since the risk of stillbirth in such cases, according to her analysis, is much greater than if the father is of any other Rh genotype.

In contrast to the views of the English workers, we have during the past seven years consistently advocated the use of induction of labour as an integral part of the treatment of Rh cases where the antenatal indications suggest that the infant will be severely affected. We believe that the time is now ripe to attempt a detailed comparison of the work of both the English group and our own.

Criteria of Severity

Indications for Exchange-transfusion

Walker and Murray (1954), as a result of several years' experience, have adopted the following criteria for determining the need for exchange-transfusion: "A Coombs-positive baby with a cord Hb level of 14.8 g.% or less, and a cord Hb level of 14.9 to 17.7 g.% associated with a bilirubin level greater than 2.7 mg. per 100 ml. . . . In those cases in which the cord-blood samples were not ideal we have been guided by whichever result was available. In practice this has usually been the cord bilirubin level, and we have treated any infant with a value greater than 2.7 mg. per 100 ml. . . . If cord-blood values were not available we have relied on clinical criteria."

Our practice during the past six years has been to rely upon the antibody level in the maternal serum as determined by a carefully standardized indirect antiglobulin method (Kelsall and Vos, 1952; Watson and Vos, 1952; Kelsall, Vos, Kirk, and Shield, 1957). No baby receives treatment if the maternal indirect antiglobulin titre at term is 1/64 or less. On the other hand, we exchange-transfuse all infants where the maternal indirect antiglobulin titre at term is higher than 1/128. Our original procedure was to transfuse all infants where the maternal titre at term was 1/128. We have found more recently, however, that some infants in this category with a high cord-blood haemoglobin value and no excess antibody free in the cord serum can safely be left alone.

Indications for Induction of Labour

In order to justify the premature termination of a pregnancy some reliable criterion must be available for predicting the severity of the disease in the infant. Since at the present time no complete certainty is associated with any of the criteria used for assessing severity in Rh cases we have to rely on a statistical evaluation of whatever criterion is being considered.

Walker and Murray (1956) have shown that when a mother has had one or more stillborn infants due to Rh incompatibility the chance of stillbirth in the next Rh-positive pregnancy is approximately 80%. A previous stillbirth is suggested, therefore, as a sufficiently reliable indication to justify induction of labour in the next pregnancy.

This criterion of severity, though perfectly valid, has the disadvantage that the mother must experience at least one stillbirth in order to provide the yardstick for prognosis in the next pregnancy. In addition there are many cases where the mother has had a seriously affected but surviving child in which the chances of a stillbirth in the next pregnancy are quite high. Walker and Murray have shown for their own series that if the previous baby was very severely affected the chance of a stillbirth is almost as high as when the previous pregnancy ended in a stillbirth, and that even when