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Suicidal Insulin Poisoning with Nine Day Survival: Recovery in Bile at Autopsy by Radioimmunoassay

The discovery of insulin fifty years ago enabled doctors to successfully treat an eleven year old boy dying from diabetes mellitus and thereby ushered in a new medical era. While millions of subsequent patients were thus able to lead productive lives, others tunred this miraculous substance into self-destructive purposes, while a few attempted (and possibly succeeded in) carefully concealed homicidal undertakings. The first "murder by insulin" case, prosecuted with a conviction in Great Britain fifteen years ago, employed a laborious bioassay method to demonstrate the hormone at the site of injection [*1*]. A recent series of insulin poisonings in California was dependent on pathologi changes in the brain from anoxia (hypoglycemia) coupled with history and investigation rather than chemical measurements, although an immunoassay method was used for confirmation in the final case [*2*]. Instances of suicidal attempts by self-administered insulin have been reported with analyses performed by radioimmunoassay, now employed in clinical laboratories to measure many hormonal and therapeutic substances [*3*]. We have applied this principle from pathologic and toxicologic aspects in an effort to enhance the diagnostic capability of the forensic pathologist, who must examine and properly certify such deaths.

Case Report

A 32-year-old white male mechanic reported to work at his usual time of 6:30 am and was noted to be "shaking." At noon, he had "some sort of seizure" and was thought to be either "very drunk or very sick." He was placed in his own automobile where two bottles of rum were noticed. His employer found him unconscious at 3:00 pm, with some "dried blood" around his mouth; he did not smell of alcohol. An ambulance was immediately called and he was taken to the hospital.

The patient had been divorced for six months and at the time of these proceedings, he was considered "mentally unstable." He had threatened to commit suicide on previous occasions and had contacted a local suicide prevention bureau. He consumed moderate amounts of alcohol, but he was not taking prescribed drugs and had no other medical history. While he was in the emergency room, the diabetic ex-wife appeared with two empty 10-ml bottles of 80 units/cc each of Lente® and Regular (Squibb) insulin and five

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used syringes which were found in the patient's apartment (Fig. 1). She indicated that on one previous occasion he had pretended to take 400 units of her insulin. It was further determined that she and the patient had remained in contact, but on this date she was going to marry another man. She last saw the patient ten days prior to this time when they had a "disagreement."

The patient was initially diagnosed as having convulsive seizures in the emergency room and was administered Valium® intravenously. He remained in a coma and soon became moderately hypertensive. The initial blood sugar was 52 mg/100 ml following which he was given 50 ml of 50 percent Dextrose and water. He did not respond to this therapy and soon became more obtunded. A lumbar puncture was performed and no abnormalities were found. A tracheostomy was done and a left carotid angiogram revealed no intracranial lesions. A repeat blood sugar was less than 25 mg/100 ml and he was then given 150 ml of 50 percent Dextrose and water and admitted to the ward.

The physical examination revealed a well developed, well nourished, comatose white male. His blood pressure was 140/60, the pulse 120 and regular, the respiration rate 28, and the temperature 99.8 deg F. His pupils were dilated but reactive with no hemorrhages or papilladema observed. Rhonchi were present in the lungs but no rales were heard. The heart revealed no murmurs. The abdomen was soft with no palpable masses. Fresh injection sites were noted over the anterior lateral left thigh, but the remaining skin was unremarkable. The neurological examination revealed response only to deep pain. Doll's eyes were present. Reflexes were normally reactive and equal, but a positive Babinski was elicited. The initial laboratory data included blood sugar less than 25 mg/100 ml, blood urea nitrogen 9 mg/100 ml, amylase less than 320, potassium 3.3 meq/l and CO_2 23 meq/l. The WBC was 22,300 with 88 percent polys. Blood gas studies showed an arterial pH of 7.39 and a PO_2 of 38 mm Hg. A chest X-ray showed mild cardiomegaly but no lung

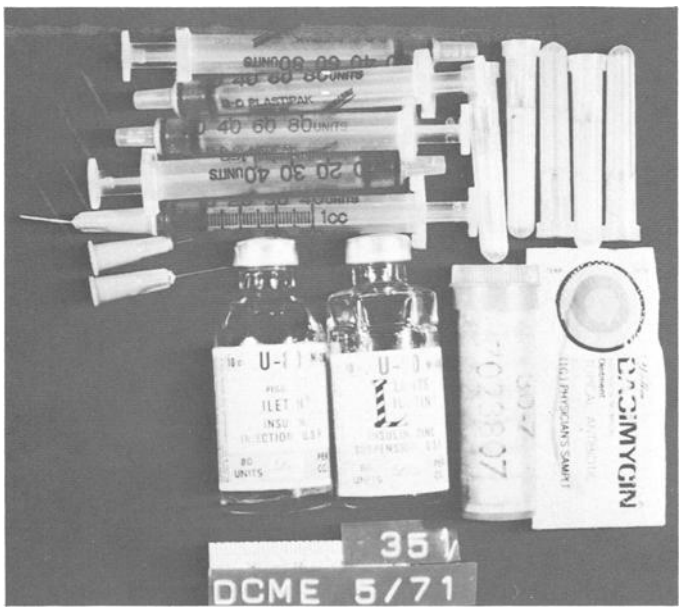

FIG. 1—*Empty insulin bottles, syringes, and antibiotic ointment recovered at patient's apartment.*

infiltrates. The electrocardiogram was negative. X-rays of the skull were normal. A toxicologic screen of the blood for common drug abuse was negative.

After his arrival on the ward, the patient was given another 50 ml of 50 percent Dextrose and water intravenously. During the next 48 hours his blood sugar was maintained above 100 mg/100 ml by a continual infusion of 50 percent Dextrose and water. There were no further hypoglycemic episodes after this period and the concentration was decreased to 5 percent Dextrose and water. His neurological status became one of flaccid paralysis with no reaction to pain and he began to have primitive gestures. An EEG on the third hospital day revealed slow diffuse waves without lateralizing discharges, indicating a metabolic encephalopathy with cortical involvement. The patient would now open his eyes and move his left arm in response to deep pain. During his hypoglycemic period, his temperature remained above 102 F, but with normal blood glucose concentrations it became stationary at 101 F. He was treated with penicillin and tetracylcine for probable aspiration pneumonitis. On the fifth hospital day, there continued to be little or no reaction to deep pain. Tracheostomy cultures revealed E. Coli and the patient was started on Gentamycin®. By the seventh hospital day the temperature rose to 105 F. The fever was treated but not controlled by aspirin, suppositories, and an ice mattress. The temperature reached 107 F on the eighth hospital day. The blood pressure dropped to 70/50 and the pulse increased to 140 on the ninth hospital day, when the patient had a sudden cardio-respiratory arrest, did not respond to saline infusions and was pronounced dead at 4:50 am. He never regained consciousness throughout his hospital stay. The final clinical diagnosis was hypoglycemic coma secondary to exogenous insulin.

The autopsy was performed 5 h after death. The anterior left thigh revealed a 2-in. linear area of skin showing several crusted abrasions 1/16 in. in diameter. Upon incision, foci of orange-tan discoloration were noted in the subcutaneous tissue. There was softening and fatty alteration of the liver, engorgement and edema of the lungs, boggy swollen kidneys, and a softened and edematous brain weighing 1585 g. Sectioning of the brain revealed ischemic necrosis in the "water shed" areas of the cerebral cortex served by the anterior and middle cerebral arteries, but no vascular obstruction was noted. The

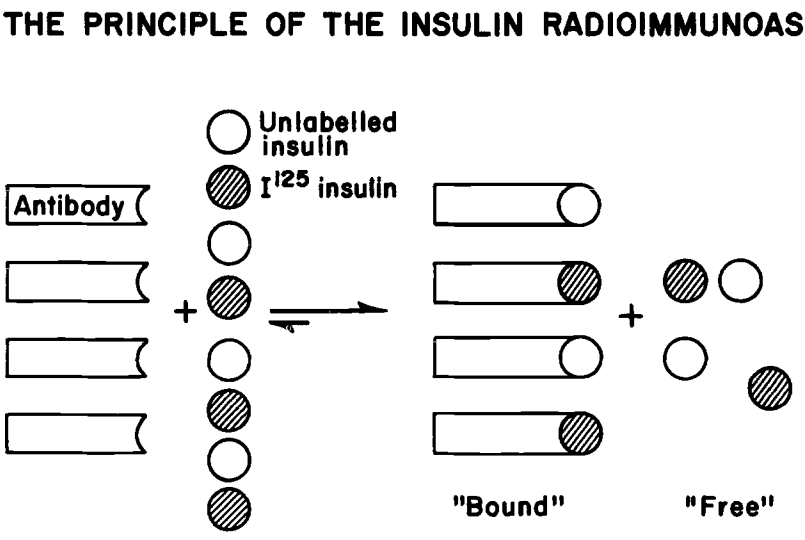

FIG. 2—*Schematic diagram illustrating the principle of competition for antibody binding between unlabelled insulin and tracer quantities of* I^{125} *insulin.*

brain stem showed pontine hemorrhages consistent with secondary pressure changes. The cerebellum and medulla were unremarkable. Microscopic examination of the brain demonstrated anoxic changes of neurons and Purkinje cells. The pancreas revealed slight enlargement of the islet cells. The other organs showed no histologic evidence of infection or other disease.

Analysis of Insulin

The blood samples frozen since the time of hospital admission and the blood and bile obtained at postmortem examination were assayed in triplicate by a modification of the double antibody technique [*4*]. This procedure uses a limited quantity of antibodies specific for insulin. The insulin in the test sample competes with trace quantities of radioactive I^{125} insulin for binding sites on the available antibody. As shown in Fig. 2, when progressively larger quantities of unlabelled insulin are added to the system, proportionately greater quantities of labelled insulin are unable to bind antibodies and thus remain unbound or free. Quantitation is achieved by the addition of known amounts of insulin and preparing a standard curve as shown in Fig. 3. The separation of the antibody-

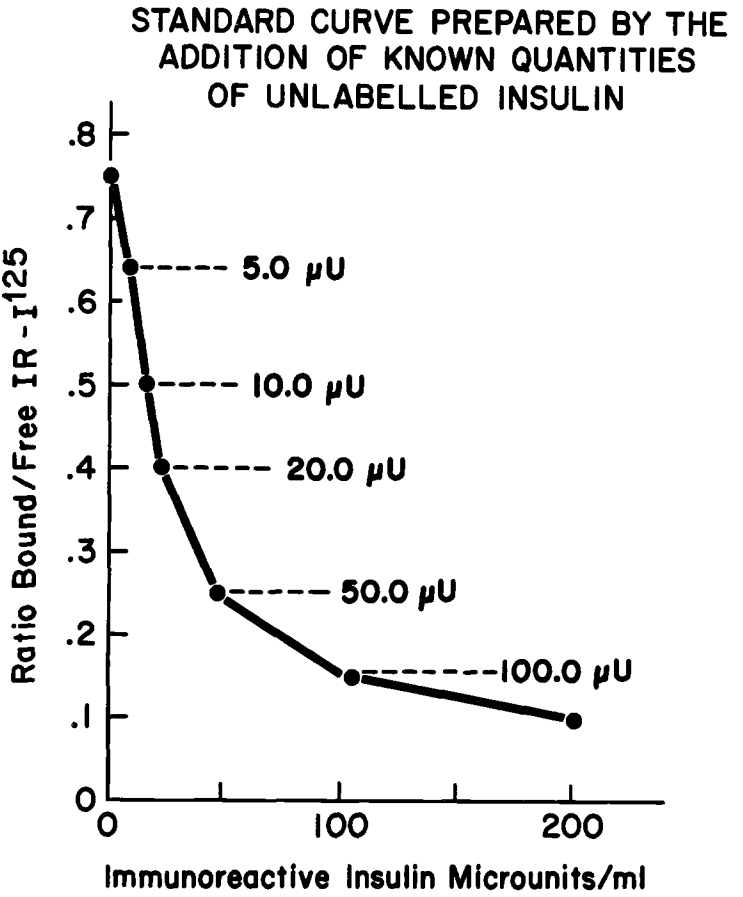

FIG. 3—*Graph illustrating preparation of a standard curve for purposes of quantitation using unlabelled insulin.*

bound and unbound insulin was achieved by a millipore filter at 4 deg C, where unbound I^{125} insulin passes through the membrane and is effectively removed. The radioactivity remaining in the membrane is therefore inversely related to the quantity of insulin present in the test sample. Measurements of larger quantities of insulin were performed by sample dilution and reassay. Values among the triplicate assays varying more than ±10 percent were subjected to subsequent reanalyses. As indicated in Fig. 3, the technique used in this assay was standardized against known concentrations of insulin ranging from 0 to 200 μunits/ml. Counting was performed by the use of a Nuclear of Chicago gamma well counter. Background activity was subtracted from each triplicate. The results of the analyses are shown in Table 1.

TABLE 1—*Analytical results of insulin analysis.*

Antemortem serum (three blood samples drawn on admission)
1830 μunits/ml
1860 μunits/ml
2010 μunits/ml
Normal Serum Insulin Level—20 μunits/ml
Postmortem specimens (nine days post-injection)
Bile: 768 μunits/ml
Serum: 42.5 μunits/ml

In order to arrive at a normal range for concentrations of insulin in the bile, samples were obtained from patients dying from a variety of conditions, including diabetes mellitus and alcoholism. The results of these determinations are shown in Table 2. In projecting the activity of Regular and Lente Insulin in the manner of supposed administration by this patient (Fig. 4), the history of symptoms shortly after arrival at work progressing to unconsciousness some hours later is consistent with the type and amount of used insulin found at the scene. A further attempt was made to estimate the amount of insulin injected by the patient calculating from the concentration determined on the admission blood samples, and the results indicate that at least 980 units of insulin could have been used.

Discussion

Prior to the discovery of insulin, it had been shown that following death, glucose was unstable in peripheral blood and that glycogen from the liver passed into the blood stream as glucose [*5*]. As it became necessary to assess the glucose concentration at the time of autopsy, one adhered to established criteria concerning postmortem alterations of glucose and related substances as they pertained to the death interval and the location of blood sampling [*6,7*]. When vitreous humor was suggested as a site capable of a more accurate estimation of glucose in the terminal state it was thought to be quite stable [*8*]. However, it was later shown that glycolysis also takes place in this fluid, and thus, sampling within a brief death interval was mandatory for a reasonable (but not always reliable) value for glucose [*9*]. It does appear that markedly elevated concentrations persist for prolonged periods following death. In any event, this is not a completely satisfactory test for terminal carbohydrate activity, and does nothing to prove whether insulin excess or deprivation was responsible for any alteration of glucose.

The widespread use of insulin was followed by establishing techniques of measurement and crude bioassay methods employing rat diaphragm were standard for many years [*10*].

TABLE 2—*Insulin concentrations in bile.*

Case	Age	Sex	PMI[a]	Alc[b]	Cause of Death	Insulin Level, μunits/ml
0906	32	M	5	neg	Insulin Poisoning	768.0
1773	53	F	30	0.20	Alcoholism	487.5
0314	16	M	12	neg	Diabetes mellitus	347.5
1433	57	F	>4	neg	Alcoholism; cirrhosis	306.25
1441	61	M	48	0.02	Chronic alcoholism (decomposed)	186.25
1186	17	M	7	neg	Multiple injuries	93.5
1013	27	F	10	neg	Islet cell tumor (Glucagonoma)	80.0
2682	65	M	>2	neg	Alcoholism; cirrhosis	51.25
1553	55	M	12	neg	Alcoholism: emphysema	56.25
2359	45	M	48	0.005	Gunshot wounds (embalmed)	41.0
1476	43	M	24	0.03	Arteriosclerotic heart disease (decomposed)	36.75
1821	24	F	12	neg.	Darvon poisoning	31.0
1857	19	M	5	unknown[c]	Insulin coma (psychiatric treatment)	29.5
2365	37	M	20	unknown[c]	Alcoholism; pneumonia	24.7
1743	21	M	14	0.08	Multiple injuries	18.0
1865	25	F	9	neg	Cardiac arrhythmia	16.2
1469	46	F	8	neg	Cirrhosis; methadone ingestion	6.5
1774	49	F	15	unknown[c]	Diabetes mellitus	0.0
1819	52	M	3	neg	Gunshot wound of head	0.0
2683	35	M	>2	neg	Myocardial infarction	0.0
2685	48	M	12	neg	Myocardial infarction	0.0

[a] Postmortem interval in hours.
[b] Blood alcohol concentration in mg/100 ml.
[c] Not analyzed.

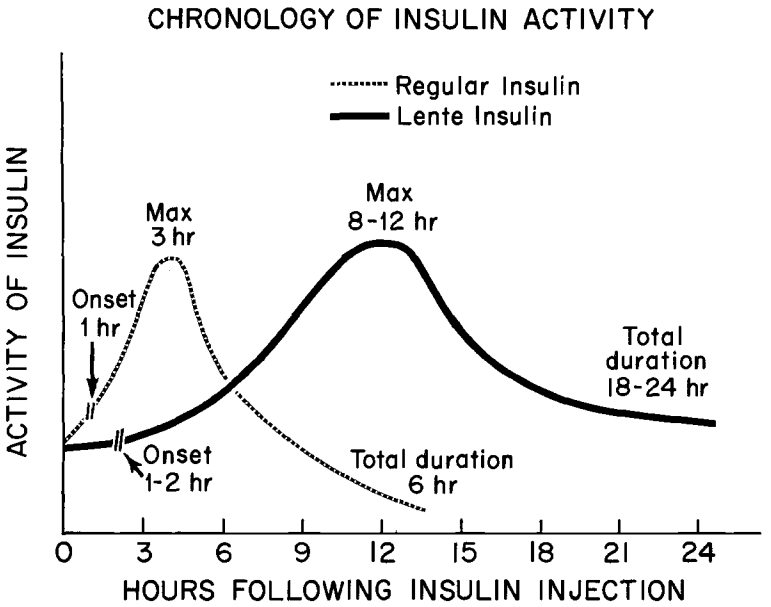

FIG. 4—*Graph depicting known activity of Regular and Lente Insulin. Patient supposedly injected at time zero and was hospitalized with blood sample obtained (and later analyzed) at ninth hour.*

With the advent of radioisotopes in medicine, these tracer substances were soon employed to analyze insulin and several other hormonal and therapeutic substances [*11*]. One obvious advantage to this technique is the fact that the amount of insulin antibodies in the patient makes no difference in the analysis. However, pro-insulin and other such forms are not distinguishable from exogenous insulin. We have examined one previous case in our laboratory, in which there was a three-day survival following self-administration of insulin, and the results showed 224 μunits/ml in the postmortem serum; antemortem blood and other postmortem tissues and fluids were not analyzed [*12*].

Insulin possesses a half-life of approximately 10 min and is 50 percent metabolized following its passage through the liver [*13*]. No information regarding actual excretion routes of insulin or its metabolites is available. Degradation is known to occur in the liver by reductive cleavage of the intrachain disulfide bonds by glutathione, and glutathione-insulin transhydrogenase [*14*]. The reduced A and B chains of insulin may then undergo rapid proteolytic degradation, be reconjugated or excreted by unknown mechanisms as suggested by this study [*15,16*].

The diagnosis of insulin poisoning in this instance is confirmed by the antemortem serum concentrations, the admission blood samples having been saved in a frozen state until they were analyzed. The autopsy blood concentration of insulin was considered to be equivocal and unable to be interpreted. Attempts to assess a normal range of postmortem serum concentrations have met with difficulty, as autolytic changes in varying postmortem intervals have yet to be determined. However, concentrations of insulin in the bile seem to persist even into periods of decomposition, but in some instances with a short postmortem interval, there was an absence of insulin.

The present case gives ample indication that, despite a prolonged survival time, injectable insulin in excess amounts is not completely metabolized in the body but rather stored in an immunoreactive form in the bile. A single instance, however, must stand the test of other case studies and experimental results, which could demonstrate contradictory findings. Further, the necessity of securing the injection site and limiting the analysis to this tissue is obviated; we have not performed analyses with this method in injection sites.

Summary

A case of suicidal insulin poisoning with nine-day survival is reported in which an excessive concentration of immunoreactive insulin measured by radioimmunoassay was demonstrated in the bile. The serum insulin concentrations on hospital admission blood specimens from this patient were approximately 100 times the normal value. Insulin concentrations in the bile following death in twenty random cases ranged from 0 to 487 μunits/ml, with two instances having detectable quantities despite decomposition. Although similar cases and experimental studies are needed to corroborate this finding, it appears that with the selection of bile as the specimen of choice at autopsy, and the employment of radioimmunoassay, an extremely sensitive and accurate techinque, the label of "undetectability" may be removed in cases of insulin overdosage even with prolonged survival times.

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