EFFECT OF ISONIAZID ON CARBOHYDRATE METABOLISM IN CONTROLS AND DIABETICS

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Following the observation that reducing substances were present in the urine of 5 out of 24 tuberculous patients receiving isonicotinic acid hydrazide (isoniazid) it was decided to investigate the nature of this reducing substance and whether isoniazid had any influence on carbohydrate metabolism. The literature contains few references to isoniazid as a reducing substance. In a report on the current status of isoniazid in the treatment of tuberculosis by the Executive Committee of the American Trudeau Society (1952) mention is made of the presence of traces of reducing substances in the urine. Their presence was not found by Benson, Steffko, and Roe (1952) or by Rubin, Lee Hassert, Thomas, and Burke (1952) in animals, nor by Robitzek and Selikoff (1952) or Elmendorf, Cawthon, Muschenheim, and McDermott (1952) in man. Selikoff and Robitzek (1952), however, mention the presence of reducing substance in the urine on one or two occasions in their series of 92 patients, but do not discuss the significance of this finding. Isoniazid contains in its molecule a hydrazine residue which provides it with a two-fold action—a reducing action and the property of combining with sugars and forming hydrazones and osazones. Preziosi (1952) reports on the interference between isoniazid and sugars in the Hagedorn and Jensen method of estimation.

Compounds chemically similar to isoniazid and other substances have been found to have an effect on carbohydrate metabolism. Izume and Lewis (1926) reported that hydrazine derivatives induce hypoglycaemia by injuring the liver and thereby interfering with the formation of liver glycogen. In addition, hydrazine, being a reducing substance, causes the urine to reduce copper solutions. Alloxan and allied compounds induce immediate hypoglycaemia associated with hepatic, renal, and pancreatic islet cell damage followed by permanent diabetes according to the extent of pancreatic islet beta-cell destruction induced (Dunn, Sheehan, and McLetchie, 1943; Koref, Vargas, Rodriguez, and Telchi, 1944). It is interesting to note that Sollmann (1948) reports that after large doses of morphine the urine often contains reducing substances which are partly morphine-glycuronic acid and partly dextrose, and that clinical doses raise the blood sugar for some hours in 10–15% of fasting individuals. This response disappears in a few weeks if the administration is continued.

Method of Investigation

The oral glucose-tolerance test was used to investigate whether isoniazid produced any disturbance in carbohydrate metabolism. The investigation was carried out in two groups of tuberculous patients, the first group consisting of

six non-diabetics to serve as "controls" and the second of six diabetics. In these 12 patients the pulmonary tuberculosis was quiescent in three and chronic in three of each group. The following tests were carried out on each patient.

Test A: an Oral Glucose-tolerance Test.—Blood-sugar levels were estimated at 0, $\frac{1}{2}$, 1, $1\frac{1}{2}$, 2, $2\frac{1}{2}$, and 3 hours. Urines were examined qualitatively for reducing substances at $\frac{1}{2}$, $\frac{3}{4}$, $1\frac{1}{4}$, $1\frac{3}{4}$, $2\frac{1}{4}$, $2\frac{3}{4}$, and $3\frac{1}{4}$ hours. The urine samples were collected at mid-points between blood samples. The test was extended from the usual two hours to three hours, so that mild diabetic trends indicated by a failure of the blood sugar to return towards normal levels, if present, could be detected. Isoniazid was then given to each patient for the next seven days.

Test B: an Oral Glucose-tolerance Test Performed after Three Days on Isoniazid.—The patients were given a dose of 5 mg. per kg. body weight and the total daily dose was divided into two equal doses administered orally at 12-hour intervals. The test began at 9 a.m., and the first dose of isoniazid for the day was given at 8 a.m.—that is, one hour before the glucose-tolerance test was started, to ensure maximal blood level of the drug. Blood and urine samples were collected at the same times as for test A. The blood plasma isoniazid levels were determined at one and a half hours after the drug was given—that is, half an hour after the start of the glucose-tolerance test—after both two and three days of isoniazid administration, to confirm that blood levels of the usual therapeutic order could be expected.

Test C: a Fasting Tolerance Test Performed After Six Days on Isoniazid.—Blood and urine samples were collected at the same times as for tests A and B, but no glucose was given. The blood plasma isoniazid levels were determined one and a half hours after isoniazid was administered.

The preparation of patients for tests A and B was as follows. The last meal was taken at 7 p.m. on the night before the test. Nothing else was eaten or drunk overnight. First thing in the morning on waking—that is, three hours before the test—a cup of weak tea with no milk or sugar was allowed. For the glucose-tolerance test 50 g. of glucose dissolved in 150 ml. of water was administered, followed by 100 ml. of water to remove the sweet taste. Blood and urine samples were collected at the times stated, with urines arranged so that blood samples were at the mid-points of urine collections.

Blood sugars were estimated from venous blood by the Folin-Wu method. Urines were examined by Benedict's method for reducing substances. If reducing substances were present their identification was attempted. Osazone tests and sugar chromatographic studies were carried out. Blood plasma isoniazid levels were estimated by the method described by Mueller and Fox (1951).

All of the six diabetics were on a mixed dose of soluble insulin and protamine zinc insulin prior to the tests, but for a week before and during the trial the P.Z.I. was discontinued and S.I. alone was given morning and evening. On the days of tests A, B, and C the morning insulin was not given until after the tests had been completed.

Results of Investigation

Dosage and Plasma Levels of Isoniazid.—The dosage and plasma levels in the controls and diabetics are presented in Table I. The total daily dosage of isoniazid varied between 250 and 425 mg. for the controls, with an average of 312.5 mg. and between 300 and 400 mg. for the diabetics, with an average of 334 mg. These doses are higher than those recommended—for example, 200 mg. daily for adults in the M.R.C. (1952) trials. In all cases the level of isoniazid was found to be in the therapeutic range. The plasma levels of isoniazid ranged from 0.3 to 0.9 mg. per 100 ml. in the controls, with an average of 0.5 mg., and from 0.25 to 0.61 mg. in the diabetics, with an average of 0.41 mg.

TABLE I.—Dosage and Plasma Levels of Isoniazid

Case No.	Sex and Age	Weight		Total Oral Daily Dose of Isoniazid	Plasma Concentrations of Isoniazid 1½ hrs. After Half the Total Daily Dose		
		lb.	kg.		Day Before Test B	Test B	Test C
Controls:					mg./100 ml.	mg./100 ml.	mg./100 ml.
1	M 25	153	69.4	350 mg.	0.8	0.4	0.7
2	M 30	187	84.8	426 "	0.3	0.6	0.9
3	M 35	124	56.2	280 "	0.3		0.5
4	F 18	118	53.5	270 "	0.3	0.4	0.5
5	F 16	134	60.8	300 "	0.5	0.45	0.9
6	F 25	109	49.4	250 "	0.6	0.3	0.3
Diabetics:							
1	M 39	143	64.9	325 mg.	0.36	0.34	0.35
2	M 38	136½	61.9	310 "	0.47	0.53	0.50
3	M 25	157½	71.4	360 "	0.41	0.32	0.40
4	F 49	175	79.4	400 "	0.5	0.42	0.61
5	F 25	137	62.1	310 "	0.4	0.25	0.39
6	F 36	134	60.8	300 "	0.33	0.31	0.48

Reducing Activity of Isoniazid.—The reducing capacity of isoniazid in terms of milligrams of sugar was estimated in order to demonstrate that the rise in blood-sugar levels after isoniazid could not be accounted for by isoniazid alone. Isoniazid added to blood in a concentration of 0.5 mg. per 100 ml. had no effect on the sugar estimation by the Folin-Wu method. It was also found that 100 mg. of isoniazid per 100 ml. had a reducing power equivalent to 37.3 mg. of glucose per 100 ml. These findings show that a rise in blood-sugar levels in these experiments cannot be ascribed to isoniazid.

Blood-sugar Curves in Controls.—The blood-sugar values for the six controls are set out in Table II, and those for the six diabetics in Table III. The average curve for the controls (Fig. 1) and for the diabetics (Fig. 2) adequately represent the pattern of any individual case. A study of the results (Table II) shows that isoniazid (1) causes a temporary rise in the fasting blood-sugar level (Table IV), (2) delays the peak of the curve, since the level at one hour is higher than at half an hour, and (3) delays the fall of the curve—the return to the fasting level not occurring, on average, until three hours.

FIG. 1.—Composite tolerance curves for six controls (non-diabetic tuberculous patients), showing tests A, B, and C.

FIG. 2.—Composite tolerance curves for six diabetic cases, showing tests A, B, and C.

The rise in the fasting level in each instance after three days' isoniazid (test B) is a temporary one, as the level is reduced again after six days' isoniazid (test C), though not to the level before isoniazid (test A), except in one case.

Blood-sugar Curves in the Diabetics.—The curves in the diabetics show much the same pattern, though in an exaggerated form. All but one (Case 4) of the six diabetic cases were severe, as shown by the standard glucose-tolerance curve. After three days' isoniazid (test B) the fasting levels and all other levels up to three hours are significantly higher. After six days' isoniazid (test C) the fasting levels have dropped a little as in the case of the controls, but not so significantly (Table IV). The levels continue to rise slowly up to three hours.

TABLE II.—Blood-sugar Values for Six Controls, Showing Tests A, B, and C

Case No.	Venous Blood Sugars (mg. per 100 ml.)						
	Time in Hours						
	Fasting	½	1	1½	2	2½	3
Test A:							
1	90	109	132	124	109	80	60
2	85	145	112	104	85	70	60
3	104	160	158	120	112	75	90
4	90	117	132	109	85	75	75
5	100	124	90	—	—	—	—
6	85	132	109	100	80	65	75
Average	92	131	122	111	94	73	72
Test B:							
1	120	189	179	141	120	117	117
2	130	137	141	141	141	112	112
3	125	141	137	141	137	137	125
4	117	141	137	132	124	129	106
5	106	106	141	141	138	116	116
6	104	112	105	117	110	105	104
Average	117	138	140	136	128	119	113
Test C:							
1	97	100	124	77	64	58	74
2	95	100	109	100	78	81	95
3	104	92	85	82	81	92	91
4	91	91	88	86	88	74	83
5	117	127	104	97	109	109	114
6	97	74	88	88	78	93	86
Average	100	97	100	88	83	84.5	90.5

TABLE III.—Blood-sugar Values for Six Diabetics, Showing Tests A, B, and C

Case No.	Venous Blood Sugars (mg. per 100 ml.)						
	Time in Hours						
	Fasting	$\frac{1}{2}$	1	$1\frac{1}{2}$	2	$2\frac{1}{2}$	3
Test A:							
1	312	418	456	510	470	462	418
2	173	271	321	385	447	424	374
3	224	288	349	394	371	357	341
4	193	297	336	249	208	186	148
5	284	422	520	584	528	454	432
6	341	432	460	482	512	422	412
Average ..	254.5	355	407	434	423	384	354
Test B:							
1	435	540	600	720	720	590	600
2	434	474	540	668	684	700	656
3	210	321	415	415	425	370	360
4	256	370	492	370	346	287	236
5	422	470	514	656	676	600	506
6	382	470	540	610	518	530	478
Average ..	356.5	441	517	573	561.5	513	473
Test C:							
1	362	432	432	432	377	432	470
2	387	402	471	437	471	483	550
3	190	223	221	246	284	269	249
4	187	170	165	162	142	135	110
5	320	340	305	330	358	363	381
6	395	370	384	370	366	335	362
Average ..	307	323	330	330	333	336	354

TABLE IV.—Variations in Fasting Blood-levels After 5 mg. of Isoniazid per kg. per Day

	Fasting Blood-sugar Levels (mg. per 100 ml.)	
	Controls	Diabetics
Before isoniazid (test A)	92±8	255±21.5
After three days' isoniazid (test B)	117±11	357±98
After six days' isoniazid (test C)	100±8.5	307±95

Variations in Fasting Blood-sugar Levels.—The variations in the fasting blood-sugar levels after isoniazid are shown in Table IV. There was a significant rise in both controls and diabetics after three days' isoniazid and a significant fall again after six days' isoniazid, though not to the level obtained before isoniazid. The figures suggest that the rise is a temporary one, but further work is required to elucidate this point.

Urine Findings.—Urine examinations were carried out on each patient in tests A, B, and C. Case 1 of the controls showed unsuspected renal glycosuria. Specimens 3–7 in test A showed the presence of reducing substances (1 plus Benedict), and these were shown to be glucose by the osazone test and sugar chromatograms. After three days' isoniazid (test B) this patient had a 2-plus Benedict's test in specimens 2–7. A trace of reducing substance was found in several of the urines from the other controls when on isoniazid dosage. The quantity was enough to turn the Benedict's solution green in these cases. The reducing substance was not glucose. The finding of traces of reducing substances in the urine during isoniazid therapy is therefore by no means constant. Urine examination in the diabetics showed that all six patients had large amounts of glucose in the urine as expected. There was moderate glycosuria in test A, increased glycosuria in test B, and a diminution of glycosuria in test C. Glucosazone tests were positive, and sugar chromatographic studies revealed that the sugar was glucose in all cases.

Behaviour of Isoniazid in Solution.—Isoniazid can be readily shown to reduce Benedict's solution. Ten isoniazid solutions of concentrations ranging from 12 to 120 mg. per 100 ml. showed a very faint reduction at 24 mg., increasing to a typical trace reaction at 120 mg. If we assume that a patient is given 300 mg. of isoniazid a day, of which 60% is excreted during that day, and that the volume of urine excreted during this period is 1,500 ml., the resulting concentration of isoniazid is 12 mg. per 100 ml. From these

considerations, concentrated specimens of urine, taken when the drug is being most rapidly excreted, may give some reduction due to isoniazid itself.

Sugar Chromatographic Studies.—A one-dimensional sugar-chromatogram showed that four runs made with pure sugar solutions, and four made with urine from tuberculous patients without diabetes but on isoniazid (controls), all showed no sugar or negligible traces of sugar. In four others done with urine from diabetic patients on isoniazid the urines showed a strong excretion of glucose only.

Summary of Results.—The findings are summarized in Tables IV and V, and show that after the administration of isoniazid (1) the fasting blood-sugar level is elevated;

TABLE V.—Changes in Oral Glucose Tolerance Test After Isoniazid

		Blood Sugar as % of Fasting Level at			
		$\frac{1}{2}$ hr.	1 hr.	2 hrs.	3 hrs. after Oral Glucose
Before isoniazid	Controls	142%	132%	102%	78%
	Diabetics	139%	160%	166%	139%
After three days isoniazid	Controls	118%	120%	110%	97%
	Diabetics	123%	145%	158%	133%

(2) the blood-sugar curve rises more slowly; and (3) the oral glucose-tolerance tests are slower to return to normal levels. The finding of trace reductions in urines from patients on isoniazid depends on the concentration of the urine and the rate of excretion of isoniazid at the time. It is thus irregular. A minimum concentration of 24 mg. of isoniazid per 100 ml. is necessary to give a reduction.

Discussion

The results indicate that the administration of isoniazid produces a temporary elevation of blood-sugar level and makes both the rise and fall of an oral glucose-tolerance test slower. As we have shown, isoniazid itself, although a reducing agent, is not responsible for the rise in blood-sugar levels. Thus a disturbance in carbohydrate metabolism may be inferred. We do not know how isoniazid impairs carbohydrate tolerance, but it may be relevant that the glucose-tolerance test in vitamin-B deficiency is similarly abnormal. Lepkovsky, Wood, and Evans (1930) reported that rats approaching their final breakdown from beriberi show a poorer glucose tolerance than their controls, which have been maintained on an identical diet but including adequate vitamin B. Lepkovsky *et al.* (1930), Bueding, Stein, and Wortis (1941), and Elsom, Lukens, Montgomery, and Jonas (1940) have all shown that in the advanced stages of aneurin deficiency glucose-tolerance curves are usually high and prolonged, indicating some impairment of the ability to utilize glucose. These abnormalities do not arise from impaired absorption, since Pachman (1941) showed that they also occur after the intravenous injection of glucose.

In the non-diabetic control patients a mean rise of blood sugar of about 20 mg. per 100 ml. over the three hours of the glucose-tolerance curve is observed, and the rise in blood-sugar levels in the diabetics was spectacular. Additional significance attaches to these findings in the treatment of tuberculous diabetics. With impaired carbohydrate tolerance insulin requirements may be increased. We have formed a clinical impression, over a short-term period on a limited number of cases, that diabetics on isoniazid are more difficult to control. The following two cases illustrate this.

Case 1

The patient, a woman aged 32, was a known diabetic of 14 years' duration. She developed a right pleural effusion in 1944 which resolved uneventfully after three months'

hospital treatment. In February, 1952, pulmonary tuberculosis was diagnosed following a two-months history of cough, sputum, and loss of weight. She was admitted to the diabetic unit at Romsley Hill Sanatorium on March 28. Her general condition was good, she was afebrile, her E.S.R. was 70 mm. in one hour (Westergren method), her sputum was tubercle-positive, and her chest x-ray film showed acute disease confined to the right upper lobe with cavitation in the right upper zone.

Her diabetes before admission had been treated with unrestricted diet and soluble insulin (S.I.) before each meal (36 units t.d.s.). Blood sugars ranged between 172 and 354 mg. per 100 ml. and glycosuria was persistent. She was stabilized on a 2,500-calorie diet and 40 units of S.I. together with 30 units of protamine zinc insulin (P.Z.I.) in the mornings, and 30 units of S.I. in the evenings. Her tuberculosis was treated with rest in bed and a three-months course of isoniazid (200 mg. daily) from April 15 to July 15 with considerable clinical and radiological improvement. Symptoms subsided, she gained 20 lb. (9.1 kg.) in weight, the E.S.R. dropped to 16 mm. in one hour, and her chest x-ray film showed considerable clearing of the disease in the right upper lobe, but there was residual cavitation for which surgery will be required. Her insulin dosage had to be raised to 44 units of S.I. and 40 of P.Z.I. in the mornings and 30 units of S.I. in the evenings in order to maintain satisfactory control of her diabetes during the period of isoniazid administration. Three weeks after isoniazid was discontinued she had her first insulin reaction and there was less glycosuria throughout the day. She developed a second insulin reaction two weeks later, and the dosage had to be reduced to 34 units of S.I. and 40 units of P.Z.I. in the mornings and 30 units of S.I. in the evenings. Control of the diabetes was more difficult to achieve during the period of isoniazid administration, and insulin dosage had to be raised. Three weeks after isoniazid was discontinued control of the diabetes was again more satisfactory, and the development of insulin reactions necessitated reduction of the insulin dosage.

Case 2

A woman aged 36 enjoyed good health until February, 1950, when diabetes was diagnosed. This was treated with 15 units of globin insulin daily. Following a haemoptysis on January 8, 1952, pulmonary tuberculosis was diagnosed, and she was admitted to the diabetic unit at Romsley Hill Sanatorium on January 19. Her general condition was fair; she was febrile (temperature 100.6° F.—38.1° C.), her E.S.R. was 46 mm. in one hour (Westergren method), her sputum was tubercle-positive, and her chest x-ray film showed extensive bilateral disease with cavitation in both lungs. Her diabetes was poorly controlled and she was stabilized on a mixed dose of 24 units of S.I. and 30 units of P.Z.I. in the mornings and 10 units of S.I. in the evenings, with a 2,500-calorie diet. Her tuberculosis was treated with a three-months' course of streptomycin (1 g. daily) and P.A.S. (20 g. daily) from January 20 to April 20, with moderate clinical and radiological improvement. Symptoms subsided, she became afebrile, the E.S.R. dropped to 15 mm. in one hour, and there was moderate clearing of the disease in both lungs. A three-months course of isoniazid (200 mg. daily) was begun on June 30, and glycosuria became more pronounced and fasting blood sugars were in the region of 200 mg. per 100 ml., so that insulin dosage had to be increased to 32 units of S.I. and 32 units of P.Z.I. in the mornings and 16 units of S.I. in the evenings. So far she has had isoniazid for two months, with some clinical and radiological improvement, but her diabetes has been more difficult to control and her insulin requirements have increased.

Summary

After isoniazid the oral glucose-tolerance test shows raised blood-sugar levels in both diabetic and non-diabetic tuberculous patients.

The insulin requirements of diabetic patients receiving isoniazid may be increased.

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REFERENCES

- American Trudeau Society (1952). *Amer. Rev. Tuberc.*, **65**, 649.
 Benson, W. M., Steffko, P. L., and Roe, M. D. (1952). *Ibid.*, **65**, 376.
 Bueding, E., Stein, M. H., and Wortis, H. (1941). *J. biol. Chem.*, **140**, 697.
 Dunn, J. S., Sheehan, H. L., and McLetchie, N. G. B. (1943). *Lancet*, **1**, 484.
 Elmendorf, D. F., Cawthon, W. U., Muschenheim, C., and McDermott, W. (1952). *Amer. Rev. Tuberc.*, **65**, 429.
 Elsom, K. O., Lukens, F. D. W., Montgomery, E. H., and Jonas, L. (1940). *J. clin. Invest.*, **19**, 153.
 Izume, S., and Lewis, H. B. (1926). *J. biol. Chem.*, **71**, 51.
 Koref, O., Vargas, L., jun., Rodriguez, F. H., and Telchi, A. (1944). *Endocrinology*, **35**, 391.
 Lepkovsky, S., Wood, C., and Evans, H. M. (1930). *J. biol. Chem.*, **87**, 239.
 Medical Research Council (1952). *British Medical Journal*, **2**, 736.
 Mueller, A., and Fox, S. H. (1951). *J. Amer. Pharm. Ass. (Sci. ed.)*, **40**, 513.
 Pachman, D. J. (1941). *Amer. J. Physiol.*, **133**, 43.
 Preziosi, P. (1952). *Rif. Med.*, **66**, 706.
 Robitzek, E. H., and Selikoff, I. J. (1952). *Amer. Rev. Tuberc.*, **65**, 402.
 Rubin, B., Lee Hassert, G., Thomas, B. G. H., and Burke, J. C. (1952). *Ibid.*, **65**, 392.
 Selikoff, I. J., and Robitzek, E. H. (1952). *Dis. Chest.*, **21**, 385.
 Solimann, T. (1948). *A Manual of Pharmacology*, 7th ed., p. 241. Saunders, Philadelphia.

SURGICAL TREATMENT OF INTRACTABLE PHANTOM-LIMB PAIN

BY

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Painful sensations in phantom limbs are common, but they vary in pattern and probably also in their mode of production. Indeed, Riddoch (1941) says that about half the people who undergo major amputations feel pain in their phantoms at some period or other. There are, for instance, the bothersome pains in the stump and phantom associated with tender and irritable stump neuromas, the pains felt in the missing limb at the site of wounding, and other fugitive pre-amputation pains. Many of these pains are transient, and if they do not disappear spontaneously are benefited by simple measures, such as resection of painful neuromas (Bailey and Moersch, 1941; Riddoch, 1941; Henderson and Smyth, 1948), procaine block of the sympathetic chain (Livingston, 1938), or even repeated percussion of stump neuromas (Russell, 1949; Russell and Spalding, 1950). But in a small minority, possibly less than 1% of amputees, the phantom pain is intense and intractable, has features which resemble causalgia, and is not relieved by these measures. It is this type of case that concerns us here.

In 1945 Falconer and Lindsay reported two cases of severe pain in a phantom upper limb which had responded to a high cervical chordotomy, after previous treatments, including resection of neuromas, sympathetic-chain block, and brachial-plexus block, had failed to give relief. Subsequently my experience has increased to 12 patients, all referred to me at the Dunedin Neurosurgical Unit in New Zealand between 1944 and 1949, and all submitted to antero-lateral chordotomy after