and subjected to paper chromatography and analysis for 6-hydroxylated indoles. Chromatography was ascending on Whatman No. 1 paper using isopropanol–ammonia–water, 10 : 1 : 1. Compounds were located with the acidic diazo reagent of Jepson[6] and with Ehrlich's reagent.

Quantitative estimations were carried out using 0·1 ml. urine, 0·9 ml. water and 5 ml. acidic diazo reagent prepared as described by Sohler *et al.*[7]. The colour was extracted into butanol and read at 415 mμ for the conjugated compounds and 530 mμ for free 6-hydroxyskatole. 'Unknowns' were compared with standard curves prepared from 6-hydroxyskatole sulphate for the conjugates and 6-hydroxyskatole for the free compound.

Chromatography of urine samples showed three 6-hydroxylated indoles to be present in the urine after the rats received 6-hydroxyskatole. The first compound (R_F 99) is free 6-hydroxyskatole[2]; the second (R_F 73) is the sulphate conjugate, while the third (R_F 15) proved to be the glucuronide of 6-hydroxyskatole. The R_F 15 material was isolated from urine by paper chromatography on Whatman 3 *MM* paper using the isopropanol–ammonia–water system. It was then eluted and chromatographed once more on Whatman 3 *MM* paper using a butanol–pyridine–water (60 : 60 : 60) solvent system. Acid hydrolysis of the purified material yielded a new spot which reacted positively with naphthoresorcinol reagent and on subsequent chromatography proved to be glucuronic acid. This spot was also obtained when the purified material was treated with 'Glusulase'—an enzyme preparation having both glucuronidase and sulphatase activity.

Fig. 1 illustrates the effect of 'Glusulase' on the 6-hydroxylated urinary metabolites. On 'Glusulase' treatment both sulphate and glucuronide spots tended to disappear while the spot of free 6-hydroxyskatole increased. 6-Hydroxyskatole would seem to be eliminated quite rapidly in the rat in the form of the free compound and the sulphate and glucuronide conjugates—the rate of excretion is maximal during the first 4 h, then levels off and is almost complete within 12 h.

Trace amounts of the glucuronide conjugate were found in human subjects who excreted large quantities of 6-hydroxyskatole sulphate. The presence of the glucuronide was established by concentrating urine samples tenfold. The concentrate was treated with urease and afterwards 200 lambda of the concentrate was spotted on Whatman 3 *MM* and run in the isopropanol–ammonia–water system. The glucuronide was detected with Ehrlich's and diazo reagent and its identity as the glucuronide was established with naphthoresorcinol reagent.

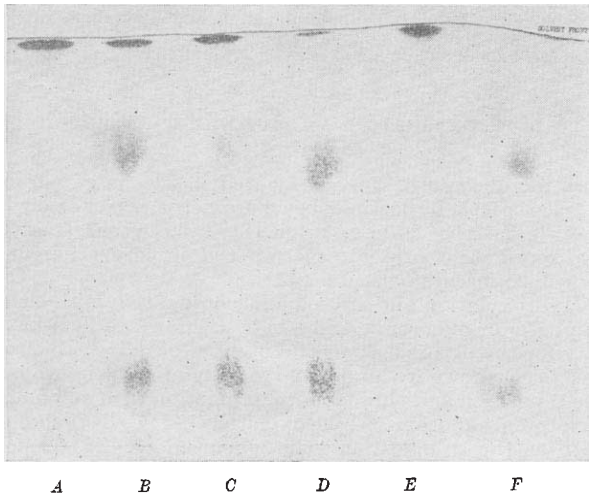

Fig. 1. Effect of 'Glusulase' on 6-hydroxylated indole metabolites in rat urine after administration of 6-hydroxyskatole. Urine samples were collected at 2-h intervals, 30 lambda of urine or its 'Glusulase'-treated equivalent was spotted. *A*, 2-h urine treated with 'Glusulase'; *B*, 2-h urine; *C*, 4-h urine treated with 'Glusulase'; *D*, 4-h urine; *E*, 6-h urine treated with 'Glusulase'; *F*, 6-h urine. Solvent system, isopropanol–ammonia–water (10 : 1 : 1)

Conjugation with glucuronic acid would seem to be an alternate pathway for the elimination of 6-hydroxyskatole acting in both rat and man.

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ARTHUR SOHLER

Neurochemistry Section,
New Jersey Bureau of Research in Neurology and Psychiatry,
c/o New Jersey Neuro-Psychiatric Institute,
Princeton, New Jersey.

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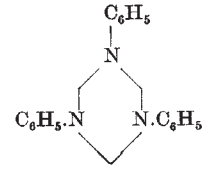
Oral Hypoglycaemic Activity of an Anhydroformaldehyde Aniline–Insulin Mixture

TWENTY-FIVE to thirty per cent of diabetic patients require insulin to remain alive and well. In the main these patients have the juvenile-onset type of diabetes mellitus and treatment for many years is normal. So far, the insulin they require can only be administered parenterally, despite many attempts in the past 40 years to circumvent the injections needed and the physical risks and psychological trauma involved[1].

It was of interest, therefore, when Ferguson[2] reported that the oral administration of a mixture of insulin and an anhydroformaldehyde–aniline (AFA) compound caused hypoglycaemia in rabbits, but the AFA compound alone was without effect. The implication that this compound allows insulin to be absorbed in a physiologically active form is of such importance that the work was repeated in our laboratories.

The AFA compound is formed by the reaction between aniline and formaldehyde, the primary product being the Schiff's base. This immediately polymerizes and under suitable conditions yields 1,3,5,-triphenylhexahydro-*s*-triazine[3], which Ferguson refers to as "anhydroformaldehyde–aniline" and gives the formula as



This can be isolated as a white crystalline material with a melting point of 141° C (ligroin).

In the present experiments, male rabbits of the New Zealand White strain (3–3·5 kg) were used, either fed or fasted for 18 h, as shown in Tables 1–3. All drugs were administered by stomach tube. Blood was taken from the marginal ear vein for measurements of the sugar-levels ('Autoanalyser' method). Samples were taken in duplicate before and at intervals after the AFA-insulin mixture or control solutions. Three separate experiments were performed.

AFA was prepared as described by Ferguson from aniline hydrochloride and commercial formalin, dried, powdered and mixed with commercial insulin ('Iletin' regular, 80 U. per ml.). This mixture was administered by stomach tube to both fed and fasted rabbits. Control solutions of

insulin, AFA alone and water alone were also given. No signs of illness were observed in any animal. Results are shown in Table 1. No hypoglycaemic effect of the mixture could be demonstrated.

Table 1. EFFECT OF ORAL AFA–INSULIN MIXTURE AND CONTROL SOLUTION ON BLOOD SUGAR OF FED AND FASTED RABBITS

Drug	No. of animals		Blood sugar mg per 100 ml. Hours after administration 0	1	2	3	4	5
AFA 200 mg	4	fed	97	107	109	102		91
	4	fasted	81	77	81		76	81
Insulin 400 U.	4	fed	97	102	102	98		94
	4	fasted	89	87	85		82	90
Water 10 ml.	4	fed	99	99	97	95		92
	4	fasted	74	75	79		76	90
AFA 200 mg plus	4	fed	91	101	95	91		88
100 U. insulin	4	fasted	80	80	80		74	85
AFA 200 mg plus	4	fed	90	91	87	84		85
200 U. insulin	4	fasted	72	76	79		76	86
AFA 200 mg plus	4	fed	88	94	91	87		81
400 U. insulin	4	fasted	78	81	81		80	86

In the second experiment, aniline was made to react with formalin in 65 per cent ethanol and the commercial insulin added after the formalin, as described by Ferguson. This suspension was administered to the rabbits without further processing. This method of preparation had been shown to be as effective as the first. The results in fasted rabbits are shown in Table 2. Thirty per cent ethanol was used as control, as this is required in the preparation to keep the reactants in solution. No signs of illness were observed in any animal. No hypoglycaemic effect of this preparation could be demonstrated.

Table 2. EFFECT OF ORAL AFA–INSULIN MIXTURE (METHOD 2) AND CONTROLS ON BLOOD SUGAR OF FASTED RABBITS

Drug	No. of animals	Blood sugar mg per 100 ml. Hours after administration 0	1	2	3	4	6
AFA plus 200 U. insulin	6	80	80	79	81	82	81
AFA alone	4	80	81	81	81	83	86
30 per cent ethanol 7 ml.	4	74	73	72	73	75	76

In the third experiment a pure sample of the anhydroformaldehyde–aniline compound was prepared according to the method of Miller and Wagner[4]. Measurements of its melting point and microanalysis give results which are similar to those with the compound prepared by the first method of Ferguson. Results are shown in Table 3 of the effect of this compound on the blood sugar-levels of fasted rabbits.

Table 3. EFFECT OF ORAL AFA–INSULIN MIXTURE (METHOD 3) AND CONTROLS ON BLOOD SUGAR OF FASTED RABBITS

Drug	No. of animals	Blood sugar mg per 100 ml. Hours after administration 0	1	2	3	4	6
40 mg AFA plus 800 U. insulin	4	68	73	67	71	69	72
200 mg AFA plus 400 U. insulin	4	79	80	80	82	81	83
200 mg AFA alone	4	75	77	78	78	74	80
2 g AFA alone	4	72	71	70	71	70	72

These results clearly show that under our experimental conditions, the anhydroformaldehyde–aniline compound as described by Ferguson does not allow insulin to be absorbed in a physiologically active form. There are two aspects of the work of Ferguson, however, which require amplification.

First, he states that fasting rabbits, before administration of the AFA–insulin complex, have blood sugar-levels of 120–140 mg per 100 ml. This is far in excess of any levels we have seen in starved rabbits. Secondly, in the second method for the preparation of AFA–insulin, ethanol was administered with insulin. It has already been shown that 20 per cent ethanol facilitates the absorption of insulin[5,6]. In a small pilot experiment in four fasted rabbits, we were able to show that ethanol in the concentration used in our second experiment (30 per cent), when given with insulin by stomach tube, causes a small but significant fall in blood sugar-levels after 1 h ($P < 0{\cdot}02$). This is not seen with insulin or ethanol alone. However, this effect of ethanol could not explain the reported hypoglycaemia produced by the first preparation of the AFA compound.

Our results do not support the claim that anhydroformaldehyde–aniline compounds facilitate the absorption of insulin in a physiologically active form.

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JOHN K. WALES*
PETER BRADLEY*
FREDERICK W. WOLFF*

Division of Clinical Pharmacology,
New York Medical College,
Flower and Fifth Avenue Hospitals,
New York.

* Present address: G. Hyman Research Building, Washington Hospital Center, 110 Irving Street, N.W., Washington, D.C.

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PHYSIOLOGY

Velocity and Force of Some Karate Arm-movements

A SPECIAL aspect of Karate (a Japanese technique of self-defence and, in competitions, a game with very strictly regulated rules in which the partners are not allowed to strike each other) can sometimes be seen as a demonstration: the Karateka breaking bricks, tiles or blocks of wood with a strike of his bare hands, feet, fingers or forehead[1], the object being supported only at its ends.

The principles of the dynamics of a suddenly applied force on a supported beam are applicable here. When a falling body strikes a beam supported at its ends with a certain velocity the beam will break if the force per unit area due to the collision is greater than the maximum stress-resistance of the material of the beam. Thus one might expect that the man who breaks a brick or other solid object with a stroke of his bare hands or feet will: (1) Develop a great velocity of movement of the mass of his arm or leg at the moment of the impact. (2) Keep a very hard and relatively small contact area between hands or feet and the object so as to concentrate the force of the impulse on a small surface. (3) Have a great determination and courage to strike powerfully at a hard object with his bare hands or feet. These three 'actions' may be singled out or work together.

We performed some studies on the velocity of some Karate movements (striking) and on the reaction force when breaking objects with the bare hand. The velocity of movement was calculated from photographs taken with the aid of a calibrated stroboscopic lamp. The movements, the velocity of which was measured, were principally accomplished in a plane parallel to the photographic plate. The force was measured with a strain-gauge device on which the objects and their supports were placed. The objects were supported at their short ends. The subjects were well-trained Karatekas (subject No. 2, for example, is one of Japan's best Karate men). They performed their movements in the usual way, without being impeded by the measuring devices. Special attention should be given to the way the Karateka holds his hand when he is going to break a brick or a plate. Breaking a brick is done with a hyper-dorsiflexed and pronated hand, the fingers almost stretched. During the impact the place of contact is the area over the pisiform bone of