its origin in the liver. Preliminary saline extraction of the factor was made by soaking liver slices (about 2 mm thick) of the shocked adrenalectomized dogs in a physiological saline solution of equal volume (4° C, 24 h). Saline washes cleared of cellular debris by centrifugation were tested, like the aforementioned serum. Only the saline extracts from the shocked animals induced an immediate fall in blood pressure, just as the serum from the shocked animals did. Thus the shock-inducing factor seemed to be liberated from the shocked liver. However, being heat-unstable it differed from VDM[6]. Thus the effectiveness of glucocorticoids in preventing endotoxin shock seems to depend on their ability to suppress the hepatic responses induced by endotoxin, such as glycogen depletion and liberation of the shock-inducing factor.

The shock in adrenalectomized dogs which could be induced even by small amounts of endotoxin differs in its mechanism from the well-documented immediate and transient falls in blood pressure observable only in this animal species with massive doses of endotoxin[7]. It seems to correspond to the late progressive shock which follows thereafter in intact dogs. It is really in this phase of shock that the effect of glucocorticoid pretreatment can be expected[2]. Whether the shock-inducing factor here demonstrated participates in endotoxin shock in other species in the absence of glucocorticoids remains to be elucidated. In rabbits, for the reasons mentioned here, the part played by this factor might be negligible. This might explain why susceptibility to endotoxin is not so much enhanced after adrenalectomy in this animal as in dogs.

Tokuro Fukuda
Takashi Kobayashi
Mitsuo Okada

Department of Physiology,
Chiba University School of Medicine,
Chiba, Japan.

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Action of Enzymatic Inhibitors (Potassium Cyanide and Sodium Fluoride) on Muscle Fibre Sensitivity to Potassium Ion

The experiments reported by Carey and Conway[1] and by Conway[2] on skeletal muscle, and by Hodgkin and Keynes[3] on nerve fibre, show that ionic exchanges, especially sodium and potassium transfer, are related to aerobic metabolism in the living tissue. Therefore, metabolic inhibitors such as cyanides modify these exchanges between cell and surrounding medium by decreasing Na^+ efflux and K^+ influx.

It has been suggested that a shift of ionic exchanges when inhibitors (cyanides or fluorides) are added to the bath could consequently modify some pharmacological effects such as potassium contracture of striated and smooth muscle.

Frog rectus abdominis. We have observed that contraction of the isolated rectus abdominis of the frog produced by potassium chloride is considerably increased by addition of very low doses of potassium cyanide to the Ringer solution in which the muscle is immersed.

The rate of increase of contractions depends on the respective doses of potassium chloride and of the inhibitor. The concentration of cyanide was maintained constant at 2×10^{-4} (3 mM) and potassium chloride concentration varied from 0·5 to 2·5 $\times$ 10^{-4} (6·7–33·7 mM). For the

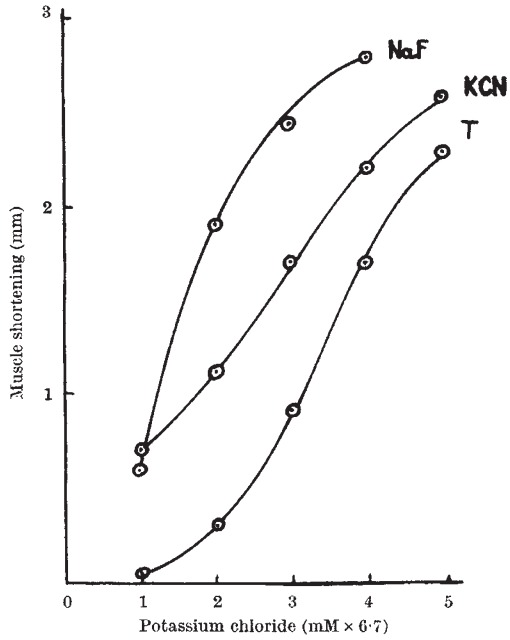

Fig. 1. Effects of sodium fluoride (4 mM) and potassium cyanide on the height of contractions produced by different doses of potassium chloride on frog rectus abdominis

lower concentrations of potassium chloride, increased contraction by cyanide may reach 10–20 times the initial contraction height.

Repeated experiments have shown that the degree of sensitization depends on the incubation period of potassium cyanide in the preparation. No effect has been observed for short periods of contact (15 sec), but sensitization takes place within 30 sec and reaches a maximum value for 60-sec contact. It is abolished after 5-min incubation.

Similar experiments were performed with sodium fluoride. At a concentration of 4 mM, very close to that used for potassium cyanide, sodium fluoride strongly potentiates potassium contraction of frog rectus abdominis; but the maximum potentiation can be obtained

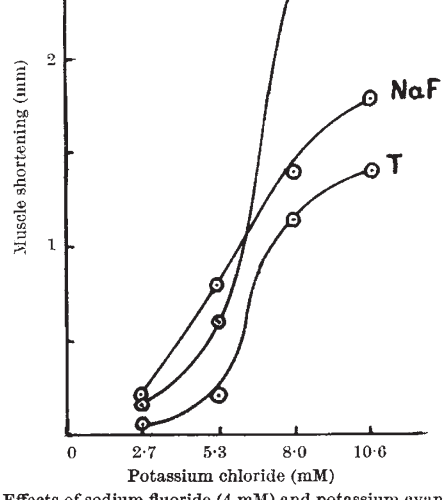

Fig. 2. Effects of sodium fluoride (4 mM) and potassium cyanide on the height of contractions produced by different doses of potassium chloride on guinea-pig ileum

only after an incubation period of 5 min. The potentiation is even stronger than that obtained with potassium cyanide. The contraction height is 3–6 times higher than the control instead of 1·9–3·7 times with cyanide, for potassium chloride concentrations ranging from 1 to 1·5 g per 1,000 (13·5–20 mM).

Fig. 1 curves describe muscle shortening (in millimetres) as a function of potassium chloride concentration in the bath, in normal conditions without any inhibitor, with cyanide and with fluoride.

Guinea-pig ileum. We observed that both enzymatic inhibitors previously used have the same influence on smooth muscle fibre potassium contracture as well as on skeletal muscle fibre.

Our experiments were performed on guinea-pig ileum, which is more sensitive than frog rectus abdominis: we obtained the same shortening with doses of potassium chloride 3–4 times weaker than in the previous experiments. Furthermore, the smooth muscle is more sensitive to the inhibitors, active doses of which did not exceed more than 1 per cent of those used on rectus abdominis. Also, it has been possible to reduce incubation period to 30 sec. So, in 0·03-mM potassium cyanide Tyrode, guinea-pig ileum shortening by potassium chloride is increased three-fold at a salt concentration less than 5·3 mM and two-fold at higher concentrations. At low potassium chloride concentrations the results are even more obvious with sodium fluoride, but at higher concentrations the potentiation is only 1·2–1·3 times.

We tried other enzymatic inhibitors with the same method: 2,4-dinitrophenol, sodium iodoacetate, sodium nitrite. No definite conclusion can be drawn from these later experiments because the results were irregular.

As a whole, the results reported here show that enzymatic inhibitors are able to sensitize smooth and striated muscle fibres to contracture produced by potassium chloride, by influencing either cellular oxidation phenomenon (cyanide) or glycolysis (fluoride).

GUILLAUME VALETTE
KEMAL OZAN

Faculté de Pharmacie,
Université de Paris.

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PHARMACOLOGY

A New Antiviral Agent: 4-Bromo-3-methylisothiazole-5-carboxaldehyde thiosemicarbazone, M and B 7714

STIMULATED by reports of the antiviral activity of some thiosemicarbazones[1], and particularly of the activity against neurovaccinia of isatin β-thiosemicarbazone[2,3], we sought to enhance the activity of these compounds by structural modification. Thus we synthesized *N*-ethylisatin β-thiosemicarbazone independently of, but somewhat later than, Bauer and Sadler[4]. At the same time, we had been making a systematic study of the chemistry and chemotherapeutic properties of derivatives of the new monocyclic ring system, 1,2-thiazole (isothiazole), and we observed that 3-methylisothiazole-5-carboxaldehyde thiosemicarbazone (M and B 7453) also protected mice infected intracerebrally with neurovaccinia. The relatively high toxicity of this compound (acute LD_{50} = 0·7 mg/g orally in mice) led us to examine related thiosemicarbazones, one of which, 4-bromo-3-methylisothiazole-5-carboxaldehyde thiosemicarbazone (M and B 7714), is considerably less toxic (acute LD_{50} = 4·3 mg/g orally in mice).

4-Bromo-3-methylisothiazole-5-carboxaldehyde thiosemicarbazone is a yellow crystalline solid, m.p. 228°–230° (decomp.)[5]. It is less than 0·1 per cent w/v soluble in water at 37°, but dissolves as a salt in alkaline solutions.

In mice infected intracerebrally with neurovaccinia (*IHD* strain) and dosed orally once daily for four days, a marked protection was observed (Table 1).

Table 1. THERAPEUTIC ACTIVITY OF M AND B ADMINISTERED ORALLY TO MICE INFECTED INTRACEREBRALLY WITH NEUROVACCINIA

	Daily dose mg/kg (× 4)	Mean survival time (days)	Animals surviving at 10 days
Controls	Nil	4·6	0/30
M and B 7714	500	9·4	22/30
M and B 7714	250	9·0	18/29
M and B 7714	120	8·0	12/29

Table 2. EFFECT OF DELAYED DOSAGE WITH M AND B 7714 ON THE SURVIVAL OF MICE INFECTED INTRACEREBRALLY WITH NEUROVACCINIA

	Dose mg/kg	Delay before treatment (days)	No. of doses given	Median survival times	Animals surviving at 14 days
Controls	Nil	–	–	4·8	0/10
M and B 7714	1,000	0	4	12·7	8/10
M and B 7714	1,000	1	3	13·2	9/10
M and B 7714	1,000	2	2	11·1	7/10
M and B 7714	1,000	3	1	8·4	4/10

Protection was still observed when treatment was delayed (Table 2). Surviving treated animals were immune to challenge from 10^5 LD_{50} infecting doses of virus. During treatment, the circulating virus titre was below that in the infected, untreated, controls. The degree of meningo-encephalitis was less after treatment.

The compound has been tested against variola major in baby mice and found to be active[6].

M and B 7714 also shows high activity in rabbits infected intranasally with rabbit pox (Utrecht strain), but is inactive against influenza, encephalomyocarditis (Columbia *SK*), and rift valley fever viruses.

Details of a fuller laboratory investigation, including toxicological studies, and the results of an extended therapeutic and prophylactic trial will be published in due course.

R. SLACK
K. R. H. WOOLDRIDGE
J. A. MCFADZEAN
S. SQUIRES

Research Laboratories,
May and Baker, Ltd.,
Dagenham, Essex.

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Distribution of Homovanillic Acid in the Human Brain

HOMOVANILLIC acid (3-methoxy-4-hydroxyphenylacetic acid, HVA) is formed by the action of the enzymes monoamine oxidase and catechol-*O*-methyl-transferase as a final product of dopamine metabolism, and its occurrence in urine has been described[1–6]. From other investigations[7,8] it may be concluded that in experimental conditions the HVA level in the brain tissue reflects the dopamine turnover, although with a certain delay. It also seems likely that for the normal brain tissue a similar statement may be made. The actual HVA concentration in the normal brain tissue, therefore, might be a suitable index in the evaluation of the local dopamine turnover. Sharman[9] has recently demonstrated the presence of HVA in the caudate nucleus of animals of several species. This seems consistent with the high dopamine concentration in this brain area[10]. Detailed investigations have been performed on the local distribution of dopamine in the human brain[11–15]. This account describes the occurrence and local distribution of HVA in the human brain.

Human brains were selected in the autopsy room, from patients not having suffered from cerebral or mental