EXPERIMENTAL DEGENERATION OF THE RETINA*

V. FASTING AND METABOLIC ACCELERATORS IN DEGENERATION PRODUCED BY SODIUM FLUORIDE

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SODIUM fluoride, a known inhibitor of glycolysis, has been tried without success by Karli (1954), Babel and Ziv (1956), and Sorsby and Nakajima (1958a) in attempts to produce retinal degeneration in the rabbit. These observers had all used other inhibitors of glycolysis in essentially exploratory experiments and their negative results for fluoride and other agents were based on small series. Degeneration of the retina in the rabbit was, however, obtained by Sorsby and Nakajima in five out of 24 rabbits treated with a single intravenous injection containing both sodium fluoride and sodium cyanide; they used this combination on the assumption that interference with both glycolysis and respiration (cyanide is an inhibitor of respiration) might produce degeneration when interference with glycolysis only was ineffective. As several other inhibitors of glycolysis and of respiration used together failed to produce retinal damage, and the effect of the combined use of sodium fluoride and sodium cyanide was obviously not constant, the question was posed whether the positive results obtained with fluoride and cyanide in combination were indeed due to the simultaneous interference with both glycolysis and respiration, or were merely occasional effects seen with either fluoride alone or cyanide alone. The present study carries these investigations further by a fuller assessment of these two agents separately, both in the normal rabbit and in the rabbit with induced derangements of carbohydrate metabolism.

Material and Methods

Dutch rabbits were used throughout. All administrations were given intravenously, the quantity of solution injected being generally about 5 to 6 ml. Ophthalmoscopic control was carried out daily, and histological control at the end of the experiment in most cases. In the course of the work with fluoride it was found that young rabbits tolerated injections better than fully-grown animals. Most of the work recorded here was therefore carried out in animals 4 to 6 months old, weighing 3 to 4 lb.

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Results

(1) Sodium Fluoride (Table I, *i* and *ii*)

Single Injections.—A total of 115 rabbits was treated with a single intravenous injection of sodium fluoride, (Table I, *i*), and seventeen of the 94 survivors showed retinal lesions. With a dose of 50 mg./kg., the losses were not too crippling and there was little to suggest that the higher doses gave any greater incidence of positive results. In this series, about 15 to 20 per cent. of rabbits therefore suffered retinal damage from a single injection.

Repeated Injections.—Of the rabbits that showed no response to a single injection, 27 were given repeated injections of 50 mg./kg., at weekly intervals (Table I, *ii*). Of the 27 which received a second injection, two showed retinal lesions and of eighteen that had three injections one gave a response. No retinal lesions developed in six rabbits which received a fourth and fifth injection and in three and two rabbits which received a sixth and seventh injection respectively. Some of these animals were kept under observation for some 3 months to exclude the possible development of delayed lesions.

TABLE I
FREQUENCY OF RETINAL LESIONS PRODUCED BY INTRAVENOUS
INJECTION OF SODIUM FLUORIDE AND SODIUM CYANIDE IN THE RABBIT

Agent	No. of Injections*	Dose (mg./kg.)	Number Treated	Number died	Number of Survivors	
					With Retinal Lesion	Without Lesions
(i) Sodium Fluoride	1	65	6	2	2	2
		60	9	4	1	4
		55	2	0	0	2
		50	75	12	12(a)	51
		40	23	3	2	18
(ii) Sodium Fluoride (Rabbits not responding to single injection)	2	50	27(b)	2	2	23
	3(c)		18(b)	2	1	15
	4		6	0	0	6
	5		6	2	0	4
	6		3	0	0	3
(iii) Sodium Cyanide and Sodium Fluoride	1	0.37—0.5	42(d)	7	7	28
		37—65				
(iv) Sodium Cyanide	3	0.5	13	1	0	12

(a) One animal showed a lesion in one eye only.

(b) One of these animals is the rabbit that developed a retinal lesion in one eye from a single injection. Further injections neither enhanced this lesion, nor precipitated a lesion in the other eye.

(c) One further animal had received a dose of 60 mg./kg. and was given two more doses without developing a retinal lesion.

(d) This series includes the 24 cases with 5 positive results recorded previously (Sorsby and Nakajima, 1958), i.e. there were 2 further positive results in 11 additional survivors.

* At weekly intervals, where more than one injection was given.

(2) Sodium Fluoride and Sodium Cyanide (Table I, *iii*)

In addition to the five positive responses in 24 rabbits previously recorded, there were two more responses in a further eleven rabbits treated. The incidence of positive results is therefore of very much the same order as that given by sodium fluoride alone.

(3) Sodium Cyanide (Table I, *iv*)

Of thirteen rabbits treated with sodium cyanide 0.5 mg./kg., none showed any retinal lesions. Two further injections at weekly intervals were given (with a loss of one animal from a second injection) without any retinal response.

(4) Facilitating Effect of Hypoglycaemia induced by Fasting and Insulin (Table II, *i*, overleaf)

Hypoglycaemia with a blood sugar level of about 30–40 mg./100 ml., is readily obtained in the rabbit by the subcutaneous injection of insulin 3 units/kg., after preliminary fasting for 24 hours. If sodium fluoride acts as an inhibitor of glycolysis, it was thought that by reducing the blood sugar available to the tissues to the lowest point compatible with life, the effect of the fluoride on the retina, which is particularly dependent on glucose, might be enhanced. As can be seen from Table II (*i*), this appeared to be the case. When 28 rabbits that had proved resistant to sodium fluoride (23 at 50 mg./kg., and five at 40 mg./kg.) were made hypoglycaemic and given a further injection of fluoride, fourteen now showed retinal lesions on ophthalmoscopic examination. A somewhat similar result was obtained with thirteen rabbits that had proved resistant to treatment by sodium fluoride and sodium cyanide combined; here five animals developed retinal lesions. In contrast, fasting and insulin did not precipitate retinal lesions in twelve animals treated with sodium cyanide alone.

(5) Facilitating Effect of Fasting Alone (Table II, *ii*; Table III, overleaf).

Table II (*ii*) suggests that the effect observed from insulin hypoglycaemia following on fasting is also obtained by fasting alone. Of seven animals resistant to fluoride, four showed a retinal lesion when a second injection was given after 24 hours fasting (though no such facilitating effect was observed with the five animals treated similarly with sodium fluoride and sodium cyanide in combination—possibly because of inadequate dosage).

That fasting and not hypoglycaemia is indeed the facilitating factor is shown by the data in Table III. Of 72 rabbits fasted for 24 hours and treated with sodium fluoride in doses of 40–60 mg./kg., 52 survived, and of these 33 showed retinal lesions—a striking contrast to seventeen out of 94

TABLE II

FREQUENCY OF RETINAL LESIONS PRODUCED BY INTRAVENOUS INJECTION OF SODIUM FLUORIDE, SODIUM CYANIDE, AND THE TWO COMBINED, IN RABBITS SCREENED FOR A POSITIVE RESPONSE AND THEN PRE-TREATED BY HYPO-GLYCAEMIA AND BY FASTING

Pre-treatment of Screened Animals	Agent	Dose per Injection (mg./kg.)	No. of Injections given in Screening	No. of Screened Animals Used	No. with Retinal Lesion	No. without Retinal Lesions
(i) 24 hrs Fasting 3 u/kg. Insulin Further Injection of Agent after 90 min.	Sodium Fluoride	50	1	14	8	6
		50	2	7	4	3
		50	3	2	0	2
		40	1	5	2	3
	Sodium Fluoride and Sodium Cyanide	40 0.5	2 or 3	13	5	8
(ii) Further Injection of Agent after 24 hrs Fasting	Sodium Cyanide	0.5	3	12	0	12
	Sodium Fluoride	50	3	7	4	3
	Sodium Fluoride and Sodium Cyanide	40	3	5	0	5
		0.5				

TABLE III

FREQUENCY OF RETINAL LESIONS PRODUCED BY INTRAVENOUS INJECTIONS OF SODIUM FLUORIDE IN RABBITS THAT HAD FIRST BEEN FASTED

Period of Fasting (hrs)	Dose per Injection (mg./kg.)	No. of Injections	No. Treated	No. Died	Retinal Lesion in Survivors		No. without Initial Response Treated with Two Further Injections	No. Died	No. with a Retinal Lesion
					+	—			
24	60	1	20	8	9	3	2	1	0
	50	1	33	6	19	8	8	2	0
	40	1	19	6	5(a)	8	9(b)	2	0
	30	1	9	2	0	7	3	0	0
48	50	1	12	6*	6	0			
	50	1	10	1	4	5			
12	50	1	12	0	2	10			

(a) One animal showed a lesion in one eye only.

(b) One of these animals is the rabbit that developed a retinal lesion in one eye from a single injection. Further injections neither enhanced this lesion, nor precipitated a lesion in the other eye.

* Experiment carried out during a cold spell.

treated with fluoride without preliminary fasting recorded in Table I (i). This series is, however, similar to the series in Table I, in that repeated injections administered to the nineteen resistant animals—resistant to fasting and fluoride—were not very productive of retinal damage. It would also appear that doses below 40 mg./kg., are ineffective even after the facilitating effect of 24 hours fasting.

Variation in Fasting Time.—Two batches of twelve and ten animals respectively were submitted to 48 hours fasting, and one batch of twelve to 12 hours fasting, before being given an intravenous injection of sodium fluoride 50 mg./kg. In the first batch, six died within 48 hours during an exceptionally cold spell of weather; the six survivors all showed retinal lesions. In the second group of ten, there was one fatality and four of the nine survivors showed retinal lesions. Of the twelve rabbits fasted for 12 hours before treatment, only two showed retinal lesions (Table III).

(6) Effect of Other Induced Metabolic Disturbances (Table IV)

(i) **Sugar Depletion by Insulin.**—The effect of insulin in rabbits not subjected to preliminary fasting is very variable. With doses of 5 u./kg., a lethal hypoglycaemia is rare. Generally the blood sugar declines markedly, sometimes after an initial rise; one hour and a half after subcutaneous injection levels as low as 30 mg. were not uncommon, whilst levels of 40–60 mg./100 ml. were frequent. As can be seen from Table IV (i), 32 animals were subjected to subcutaneous injection of insulin 5 u./kg., and 90 minutes later were given 50 mg./kg., sodium fluoride. Of the 23 survivors, only five showed an ophthalmoscopic lesion. A second treatment of the eighteen irresponsive rabbits (and one rabbit that had a lesion in one eye only) produced three fatalities and four positive retinal responses, whilst seven surviving and resistant animals responded to a third treatment with three positive results, three deaths, and one residual resistant rabbit (which also remained resistant to a fourth treatment). In all, insulin depletion of sugar therefore produced

TABLE IV
FREQUENCY OF RETINAL LESIONS PRODUCED BY INTRAVENOUS INJECTION OF SODIUM FLUORIDE IN RABBITS SUBJECTED TO PRE-TREATMENT OTHER THAN FASTING

Pre-treatment	Agent	Mode of Injection	Dose per Injection per kg.	No. of Animals Treated	No. Died	No. with Retinal Lesions	No. given a Second Treatment	No. Died	No. with Retinal Lesions	No. given a Third Treatment	No. Died	No. with Retinal Lesions
(i) By Drugs given 90 min.* before Injection of Sodium Fluoride (50 mg./kg.)	Insulin	Sub-cutaneous	5 u.	32	9	5(a)	19(b)	3	4	7	3	3
	Dinitro-phenol	Intra-venous	20 mg.	22	3	9	10	3	2	—	—	—
	Tri-iodo-thyronine	Intra-venous	3 mg.	9	0(c)	4						
	Synthalin B	Intra-venous	3 mg.	12	4	4	3	—	2	1	—	—
(ii) By Diabetes Induced before Injection of Sodium Fluoride (50 mg./kg.)						No. not Diabetic after Sixth Day			No. of Diabetic Animals treated with Sodium Fluoride			
	Alloxan	Intra-venous	150	25	8	5			12			3

(a) One animal showed a lesion in one eye only.

(b) One of these animals is the rabbit that developed a retinal lesion in one eye from a single injection. Further injections neither enhanced this lesion, nor precipitated a lesion in the other eye.

(c) Five rabbits—three with retinal lesions and two without—died fairly suddenly on the fourth and fifth day.

* Sodium fluoride was administered 6 hrs after tri-iodo-thyronine.

lesions in twelve out of 23 animals, though repeated treatments were needed to reach this total. There appeared to be no relationship between the degree of relative hypoglycaemia and the susceptibility to fluoride. The positive, no less than the negative results, were scattered over the whole range of blood sugar levels observed.

(Insulin zinc protamine did not appear to have any advantages over soluble insulin. Eight rabbits were treated with subcutaneous injection of insulin zinc protamine 3 u./kg. on 3 to 5 successive days and given an injection of fluoride 2 hours after the last administration of insulin; two died and two showed retinal lesions.)

(ii) *Alloxan Diabetes*.—Of 25 rabbits given intravenous injections of alloxan 150 mg./kg. (Table IV, ii), seventeen survived for 6 days or longer; of these, five proved resistant to alloxan and the remaining twelve developed diabetes with a blood sugar in excess of 250 mg./100 ml. Sodium fluoride 50 mg./kg., was given intravenously in these twelve animals and produced retinal lesions in three. There was no direct relationship between the level of blood sugar and a positive response or lack of it.

(iii) *Dinitrophenol*.—Of 22 rabbits treated with 20 mg./kg. dinitrophenol injected intravenously, followed 90 minutes later by an intravenous injection of sodium fluoride 50 mg./kg., nineteen survived, of which nine developed retinal lesions. The ten resistant animals were given a second treatment 14 days later; seven survived, of which two developed retinal lesions. In all, of nineteen animals submitted to one or two treatments, eleven showed retinal lesions.

(iv) *Thyroid*.—Nine rabbits were given 3 mg./kg., L-tri-iodo-thyronine sodium intravenously, and 6 hours later a further injection of 50 mg./kg. of sodium fluoride. Within 3 days four showed retinal lesions ophthalmoscopically. (On the fourth and fifth days three of the rabbits with fundus lesions and two without fundus lesions died fairly suddenly.)

(v) *Synthalin B*.—Of twelve rabbits given an intravenous injection of Synthalin B (dodecamethyleneguanidine) 3 mg./kg., and 90 minutes later an injection of sodium fluoride 50 mg./kg., four succumbed. Of the eight survivors, four showed retinal reactions; three of the unresponsive rabbits were given a second treatment and two of them developed retinal lesions. A third treatment in the sole surviving resistant animal gave no result.

(7) Effect of Agents other than Sodium Fluoride injected after Fasting (Table V, opposite)

Table V (a) shows a series of agents which proved to be without retinotoxic effect; these still showed no such effects after fasting and induced hypoglycaemia. Included amongst these agents were dinitrophenol, Synthalin B,

TABLE V
LACK OF RETINAL LESIONS FROM INTRAVENOUS INJECTIONS OF SOME AGENTS OTHER THAN SODIUM FLUORIDE

Agent	First Injection					Repeat Injection					Remarks
	Pre-treatment	Dose mg./kg.	No. Treated	No. Died	Retinal Lesion in Survivors	Pre-treatment	No. Treated	Retinal Lesion			
					+ -			+ -			
(a) Agents used without pre-treatment and subsequently after induced hypoglycaemia											
Dinitrophenol	None	20	7	1	0	6	24 hrs Fasting and 3 u/kg. Insulin 90 min. before Injection	6	0	6	Three died
Dinitro-orthocresol		20	6	2	0	4		4	0	4	
Sodium selenite		15	11	0	0	11		11	0	8	
Sodium oxalate		50	3	0	0	3		3	0	3	
Sodium p-chloromercuriben-zoate		40	6	1	0	5	All five died 3-4 days after first injection				
Synthalin B		3	7	1	0	6	Repeat experiment not done				
L-tri-iodothyronine		3	8	0	0	8	Repeat experiment not done				
(b) Agents known not to produce retinal lesions tried after fasting											
Nicotine	24 hrs Fasting	2	8	1	0	7	One Week Later after 24 hrs Fasting	7	0	6	One died
Methyl glyoxal		50	7	1	0	6		6	0	6	
Sodium azide		12.5	5	3	0	2		2	0	1	One died
Sodium iodoacetamide		37	5	2	0	3		3	0	0	Three died
A tertiary amino phenoxyalkane (a)		60	9*	0	0	9	Synthalin B 3 mg./kg. 90 min. before Injection	9	0	9	
"N.P. 207" (b)		20	8	0	0	8	Repeat experiment not done				
Sodium oxalate		50	5	0	0	5	Repeat experiment not done				

* Fasted for 48 hours
(a) 1:5 di (p-dimethylaminophenoxy) pentane.
(b) 3-chloro-10/2'-(-methyl-piperidyl-2'')-ethyl/-phenothiazine hydrochloride.

and tri-iodo-thyronine, which had been found to facilitate the effect of fluoride. Fasting was likewise found to be without effect in a series of agents previously known not to produce retinal damage (Table V b). Amongst these were carbohydrate inhibitors (Sorsby and Nakajima, 1958a), a phenoxy-alkane compound (Sorsby and Nakajima, 1958b), and a phenothiazine derivative (NP 207), which though retinotoxic to man has not given retinal lesions in the experimental animal (Wagner, 1956).

Fluoride therefore stands alone amongst the different agents tried both in its occasional effect on the retina, and in the increased frequency of response obtained after fasting or other pre-treatment.

(8) Ophthalmoscopic Appearances and Histological Findings

The retinal lesion produced by fluoride is characteristic in onset and extent. Within 48 hours the lesion is obvious in most cases; the earliest time at which

it has been observed was 6 hours after injection, and occasionally a lesion has not become manifest until after 5 days. Commonly the lesion covers a zone 1.5 disc diameters in depth and extending right across the lower half of the fundus, sparing the area immediately below the disc. The earliest signs are sharp demarcation lines delineating the extent of the lesion; within the zone delineated the retina looks oedematous. Within 2 to 3 days this oedematous zone becomes flatter and pigmented, the pigmentation being easily distinguished from other pigmentary changes normally seen in the rabbit fundus by its intense black coloration and uniform patterning throughout the affected zone. The pigment spots are generally large roundish dots, though irregular shapes may also appear. The lesion is fully established within 14 days. There are two variants to this picture. Only exceptionally is the whole of the fundus involved instead of the broad zone described. Rather more commonly, smaller areas than the broad zone are affected; such smaller areas may be single or multiple. Exceptionally, only one fundus is affected—a finding confirmed histologically.

The illustration shown in a previous publication (Sorsby and Nakajima, 1958a) represents a rather extreme variant of the lesion in that most of the fundus was affected. Ophthalmoscopically the fluoride lesion differs from the appearances observed with other substances that induce retinal damage in tending to be less extensive.

Histologically the appearances already recorded in the previous study have been found consistently in this series. There is marked destruction of the rod layers and of the pigment epithelium with slight or little involvement of the inner layers of the retina.

Discussion

(1) Place of Sodium Fluoride amongst Retino-toxic Substances

The present study adds a fifth substance to the four that have been hitherto known to produce retinal damage in the experimental animal—sodium iodate, sodium iodoacetate, dithizone, and the phenoxyalkanes. The mode of action of all five substances still remains to be established. It is possible that they do not have one common underlying effect, but that the different agents produce their damage in different ways. There is no evidence that sodium iodate acts as an enzyme inhibitor in life; the versatility of sodium iodoacetate as an enzyme inhibitor is such that it is difficult to incriminate any particular enzyme effect; the action of dithizone is obscure, and an outstanding effect is the diabetes produced from damage to pancreatic tissue; and nothing is known of any enzyme effect of the phenoxyalkanes. Sodium fluoride has some unusual features. With adequate doses all the agents hitherto known produce retinal damage constantly, or very nearly so; in contrast, sodium fluoride is effective in only some 15–20 per cent. of rabbits.

The marked increase in the incidence of positive results obtained when the rabbits were pre-treated by insulin—on the assumption that sodium fluoride as an inhibitor of glycolysis would be most effective in hypoglycaemia—proved to be due not to the hypoglycaemia but to the preliminary fasting employed. Whilst fasting itself greatly facilitated the effect of fluoride, substantial facilitation was also obtained independently of fasting by insulin and several other agents with metabolic effects: dinitrophenol, tri-iodo-thyronine, and Synthalin B (but not by alloxan diabetes). No procedure was effective in producing retinal damage constantly. It is thus obvious that both the action of fluoride and the mode of action of the enhancing factors shown in the study remain obscure, for to speak in general terms of fasting and of metabolic disturbances—or possibly of a hepato-toxic effect as is suggested by Synthalin B—is only stating the problem. Fluoride, known as an enzyme inhibitor in the series of changes that occur in the normal phosphorylating glycolysis, is a most versatile substance, and bearing on its mode of action on the retina, the following observations are relevant:

- (a) Injection of fluoride into the carotid artery of an animal produces retinal oedema [? instantaneously]. This result is recorded briefly by de Berardinis and Bonavolontà (1953).
- (b) In studies on the developing retina, vasoconstriction followed the injection of fluoride intravitreally in the living kitten; no such effect was seen in the adult cat (Ashton, Graymore, and Pedler, 1957; Pedler, 1959).
- (c) *In vitro* studies have shown that:
 - (i) Fluoride inhibits lactic acid formation and phosphorylation in retinal extracts (Kerly and Bourne, 1940; Holmes, 1940).
 - (ii) Fluoride produces oedema in the immature retina exposed to it (Graymore, 1959).
 - (iii) Histological damage and interference with glycolysis or respiration occurs only after exposure to high concentrations (Lucas and Newhouse, 1959).

(2) Nature of the Facilitating Effect seen with Fluoride

In experimental pathology there are several other instances in which fasting or the use of metabolic accelerators has a facilitating effect similar to that recorded here for fluoride degeneration of the retina. The observations deal with the effect of fasting (or of dietetic derangements) on the toxicity of some drugs, and the effects of fasting and of metabolic accelerators on susceptibility to infection, and the influence of fasting on the developing embryo.

(a) *Effect of Fasting and of Dietetic Derangements on Toxicity of Drugs.*—The literature of the end of the 19th and the beginning of the 20th century contains many references on this aspect. It was noted that fasting or malnutrition enhanced the toxicity of some drugs, and diminished that of others (*see* Hooper, Kolls, and Wright, 1921). Such findings were important clinically if only for two reasons.

In the first place it was known that chloroform was much more toxic to a fasting patient than to a patient who had received adequate nourishment, and secondly, with the advent of Salvarsan and its derivatives, there also came the recognition that arsenic was more toxic to a starving animal or to a fasted patient.

Later studies established the significance of exclusively dietetic factors—as opposed to toxic factors—in damage to the liver, a distinction emphasized by Himsworth and Glynn (1944) by the terms tropopathic and toxipathic lesions. Dietetic deficiencies came to include such clearly isolated factors as choline, methionine, the lipotropic agents generally, and tocopherol, whilst the list of toxic agents capable of producing liver damage is now considerable (see Drill, 1952; Hartroft, 1956; Himsworth, 1950).

Bearing closely on the fluoride results reported here, are the observations on liver damage by trinitrotoluene (TNT). This agent apparently presented a considerable problem during the first world war, when liver damage was a significant problem in TNT workers. Experimentally it was, however, difficult to produce lesions, only two out of ten rabbits in one particular experiment being found to show fatty degeneration of the liver (Medical Research Committee, 1917). Such experiments, repeated in rats fed on a low protein/high fat diet, gave much more consistent results (Himsworth and Glynn, 1942). Somewhat similar observations have been recorded for tetrachlorethane: dogs fasted for 24 hours respond with severe intoxication and coma to doses that are innocuous to animals on normal diet (see Wright, 1952).

(b) *Fasting and Metabolic Accelerators in Relation to Infection.*—It has been known for many years that the nutritional state—whether of the experimental animal or a patient—influences the course of an infection. Recently Dubos (Dubos, 1955; Dubos, Smith, and Schaedler, 1955) has shown the marked effect of fasting for 24–36 hours on mice exposed to *Staphylococcus aureus*, *Klebs. pneumoniae*, and *B. tuberculosis*; all animals fasted showed a high mortality to mild infections that were innocuous to normally-fed mice. Dubos believes that this effect arises from the transient production or accumulation in the tissues of fasted animals of metabolites which favour the activities of micro-organisms, for he found that he could increase the virulence of tuberculous infection in mice in a striking and consistent manner by adding to otherwise satisfactory diets certain metabolites, such as sodium butyrate, citrate, or glutarate; alcohol or lactate has no similar infection-enhancing effect. The possibility of an endocrine factor is suggested by observations showing that dinitrophenol and thyroxine added to a normal diet enhance the susceptibility of mice to infection with tubercle bacilli and staphylococci.

(c) *Fasting as a Teratogenic Factor.*—It appears that fasting during pregnancy enhances an existing tendency towards malformation in the foetus. Fasting for 24 hours on the 9th day of pregnancy in the mouse resulted in 22 per cent. of the foetuses having either cranioschisis or deformed ribs or both—against 2 per cent. in untreated mice (Runner, 1959). Foetuses were protected from the fasting effect by relatively small quantities of a variety of nutrients, such as glucose, casein, and glutamic acid.

The mode of action of fluoride remains as obscure as that of other retinotoxic agents. A simple explanation, such as one postulating interference with glycolysis, can hardly be maintained in face of the failure to obtain degeneration by other inhibitors of glycolysis. Theoretically many possibilities are open, and until further data are available the most puzzling—and most tantalizing—aspect of fluoride degeneration of the retina is its substantial dependence on the nutritional state.

Summary

(1) Unlike other inhibitors of glycolysis (with the exception of sodium iodoacetate which has many other activities), sodium fluoride produces retinal degeneration in the experimental rabbit. The effect is, however, not constant.

(2) When effective, sodium fluoride produced a retinal lesion by a single intravenous injection of doses of 40 to 60 mg./kg., generally within 48 hours. Of 115 rabbits so treated, 94 survived and seventeen showed retinal lesions. A dose of 50 mg./kg. is best; lower doses are not very effective and higher doses are more toxic without being more effective. Of 63 rabbits treated with a dose of 50 mg./kg., twelve showed a retinal lesion.

(3) Ophthalmoscopically the lesion is highly characteristic. It is rather more limited in extent than that produced by the other agents that induce experimental retinal degeneration, and is sharply delineated from the rest of the fundus. Histologically the pigment epithelium and rod layer of the retina are involved.

(4) The combined use of sodium fluoride as an inhibitor of glycolysis and sodium cyanide as an inhibitor of respiration did not increase the frequency of positive results.

(5) In rabbits not susceptible to a single intravenous injection of sodium fluoride, repeated injections produce a result only exceptionally: there were two responses in 27 animals given a second injection of 50 mg./kg., and one further response in eighteen given a third injection. In contrast, out of 23 insusceptible rabbits given an injection of 50 mg./kg. after first being made hypoglycaemic by fasting and insulin, twelve showed retinal lesions. There was no direct association between the degree of hypoglycaemia and the development of a lesion.

(6) Each of the two procedures used to produce hypoglycaemia was itself effective in increasing the incidence of positive responses to fluoride (though neither produced a retinal lesion on its own). Fasting for 24 hours before the injection of 50 mg./kg. sodium fluoride gave nineteen positive responses in 27 rabbits. Repeated pre-treatment with insulin gave twelve positive results in 23 rabbits.

(7) Pre-treatment by full doses of dinitrophenol, tri-iodo-thyronine, and Synthalin B—agents that by themselves are not productive of retinal lesions—also increased the incidence of retinal lesions to some 50 per cent. The induction of alloxan diabetes had no such effect.

(8) The facilitating effect of fasting observed with fluoride was not obtained with other substances tried.

(9) Attention is drawn to the parallel findings on the facilitating role of fasting in experimental liver damage, and of fasting and metabolic accelerators in experimental susceptibility to infection, and on the influence of fasting in inducing congenital malformations.

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