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COMMUNICATIONS

A STUDY OF THE CORRELATION BETWEEN THE
BIOCHEMICAL AND INTRA-OCULAR CHANGES IN-
DUCED IN RABBITS BY THE ADMINISTRATION OF
NAPHTHALENE*

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Introduction

THE action of naphthalene on the animal body has been studied independently, by ophthalmologists who were interested in the production of cataract, and by chemists who studied the metabolism of naphthalene as being one of the carbocyclic compounds. There is, however, an obvious opportunity for the biochemist to seek to correlate the two sides of the problem, for as I have already indicated in a previous paper (1930), the ocular lesions are in themselves of such a nature as to suggest that they may be due to a disturbance in the normal physiological balance of the body, rather than to a specific toxic action of naphthalene or of one of its derivatives. It is true that until recently, the latter view has been the one more generally accepted, and the evidence for it must be recorded (see Section II), as well as considering the former and more attractive hypothesis.

Although there is proof of the metabolism of naphthalene, so far no definite chemical toxin has been identified. On the other hand, a variation in some of the normal constituents of the blood

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has recently been reported, (Michail and Vancea, 1927, Komura, 1928). A repetition of experiments of this kind (see Section I), has shown similar variations, although the results do not altogether confirm those of previous observers, and their exact significance is not yet clear.

Section I

The Variation in certain Normal Constituents of the Blood after the Administration of Naphthalene

A preliminary investigation was made of the variation in three blood-constituents only, viz:—sugar, cholesterol and calcium, and of these only the last showed any marked change. Throughout the experiments, a close comparison was made between the chemical variations and any intra-ocular changes which developed.

1. Variation in Blood Sugar

Historical. Some interesting results have been published by Michail and Vancea (1926—27). They state that there is a hyperglycaemia half-an-hour after the administration of naphthalene (in glycerine) by any route, but that it falls again to the basal level during the course of the day. With continued dosage of 1 gm. of naphthalene per day, the basal level itself rises to a maximum in 5—7 days, *e.g.*, from 0.905 to 1.50 gm. per 1,000 c.c. and then falls to its original value in 10 or 12 days. In one case after an intra-peritoneal dose from which the rabbit died on the seventh day, they record a hyperglycaemia rising to 4.445 gm. per 1,000 c.c. Komura (1928), also records the occurrence of hyperglycaemia, *e.g.*, from 0.09 to 0.11 or 0.13 gm. per 100 c.c., beginning seven hours after the administration of naphthalene (in capsules), and reaching a maximum 30 hours later. The blood-sugar returned to its normal level in 4—5 days.

Michail and Vancea conclude from their results that the effects of naphthalene are really those of a pancreatic dysfunction, and that the resulting cataract is therefore of a diabetic type. They state that insulin will delay the formation of a complete cataract if it is administered when the lens opacities have just begun to appear, while it will prolong the life of an animal to which a lethal dose of naphthalene has been given. They do not, however, indicate what dose of insulin was employed, nor how often it was given. This fact, together with the known inconstancy in the appearance of lens lesions, and the possibility that a certain degree of retrogression might occur in any case, make it difficult to assess the value of this observation.

Experimental. Three healthy rabbits were employed, and were dosed as shown in the following table:—

TABLE I.

Rabbit.	Initial weight.	Daily dose (per os).	No. of days.
XI.	2.5 kg.	2 gm. naphthalene	21
X.	2.6 kg.	2 gm. naphthalene 3 gm. glucose	15
VI.	2.8 kg.	(a) 3 gm. glucose (b) 2 gm. naphthalene	7 4

The naphthalene was administered by means of a stomach-tube in solution in warm paraffin (approximately 8 c.c. for 1 gm.) as glycerine was considered an unsuitable medium. In one case glucose was given in addition to naphthalene, and a control experiment on the effects of glucose alone, was done. The animals remained healthy and maintained their weight throughout the experiment.

The variations in blood sugar were followed by duplicate estimations by Hagedorn and Jensen's method (1923) on:—(1) "fasting" samples taken each day before the animal was dosed (see Tables II and III A, B, C.) (2) Samples taken at intervals of a few hours to show the variation on the first two days of administration (see Tables IV A, B, C.)

The ophthalmoscopic changes were noted throughout the experiments, and these were compared with the blood-sugar values.

TABLE II.

A Comparison of the Daily Variations in Blood-sugar after Naphthalene.

Days	Rabbit XI.	Rabbit X.	Rabbit VI.	Rabbit I. (Michail & Vancea)
1	0.126 gm. %	0.092 gm. %	(a) 0.091 gm. %	0.091 gm. %
2	0.124	0.099	0.101	0.093
3	0.117	0.094	0.107	0.137
4	0.107	0.107	0.108	0.137
5	0.126	0.124	0.099	—
6	0.117	—	—	0.185
7	0.117	0.123	0.114	—
8	—	0.093	(b) 0.095	0.142
9	0.117	0.095	0.109	—
10	0.099	0.092	0.095	0.151
11	0.085	0.099	0.095	—

TABLE II.—*continued.*

Days	Rabbit XI.	Rabbit X.	Rabbit VI.	Rabbit I. (Michail & Vancea)
12	0'102	0'096	—	0'136
13	—	—	—	—
14	—	0'091	—	—
15	0'097	0'101	—	0'135
16	0'084	—	—	—
17	0'098	—	—	—
18	0'098	—	—	0'139
19	—	—	—	0'102
20	—	—	—	0'103
21	0'108	—	—	—
22	—	—	—	0'091
23	—	—	—	0'094
24	—	—	—	0'094

N.B.—The figures in the fourth column are adapted from those of Michail and Vancea (*C. R. Soc. Biol., Paris*, XCVI, i, 1457, 1927).

TABLES III A, B, C.

*A Comparison of the Variation in Blood-sugar with the
concurrent intra-ocular changes.*

TABLE III A.—Rabbit XI, given 2 gm. naphthalene daily.
(12/2/29—5/3/29.)

Days	Time of bleeding	Blood-sugar	Ocular lesions
1	11.0 a.m.	0'126 gm. %	Retina and lens clear
2	11.38	0'124	Retinal exudates began at 3
3	10.56	0'117	Increase in size and extent of exudates; lens striae developed on 4th day
4	11.26	0'107	
5	12.20	0'126	
7	10.57	0'117	Retina becoming atrophic
9	3.5	0'117	
10	12.0	0'099	Gradual advance in lens opacities
11	10.52	0'085	
12	9.40	0'102	
15	11.8	0'097	
16	4.10	0'084	
17	2.22	0'098	Immature cataract right and left; atrophy of retina, some crystals in vitreous
18	11.50	0'098	
21	10.5	0'108	

TABLE III B.—Rabbit X, given 2 gm. naphthalene and 3 gm. glucose daily. (12/2/29—26/2/29.)

Days	Time of bleeding	Blood-sugar	Ocular lesions
1	11.8 a.m.	0.092 gm. %	Retinal exudates began at 6
2	11.15	0.099	Extensive exudates, fine lens changes
3	10.37	0.094	Lenticular striae advancing
4	11.11	0.107	Development of posterior
5	12.41	0.124	cortical lens opacities
7	10.41	0.123	Gradual advance in opacity of both lenses
8	3.20	0.093	
9	2.38	0.095	
10	12.11	0.092	
11	10.35	0.099	
12	9.50	0.096	
14	11.20	0.091	Complete cataract right eye, partial cataract left
15	10.50	0.101	

TABLE III C.—Rabbit VI. (a) As normal control on daily dose of 3 gm. glucose and 16 c.c. paraffin. (12/2/29—18/2/29.)

(b) On daily dose of 2 gm. naphthalene followed by immediate access to food. (5/3/29—8/3/29.)

Days	Time of bleeding	Blood-sugar	Ocular lesions
a) 1	11.30 am.	0.091 gm. %	None
2	11.26	0.101	"
3	11.11	0.107	"
4	11.34	0.108	"
5	12.26	0.094	"
7	10.55	0.114	"
(b) 8	10.55	0.095	Retina clear
9	10.0	0.109	Small exudates at 10 a.m.
10	11.0	0.095	Increase in number of exudates
11	11.0	0.095	Increase in size of exudates

TABLES IV A, B, C.

Showing the immediate Variation of Blood-sugar after Naphthalene.

TABLE IV A.—Rabbit XI, on naphthalene.

Days	Time of bleeding	Blood-sugar	Ocular lesions
1	(1) Before dose	0·126 gm. %	Retina and lens clear
	(2) 33m. after „	0·110	
	(3) 1h.24m. „ „	0·111	
	(4) 2h.12m. „ „	0·104	
	(5) 3h.23m. „ „	0·090	
	(6) 8h.37m. „ „	0·105	
2	(1) Before dose	0·124	Retina clear
	(2) 53m. after „	0·133	
	(3) 3h.4m. „ „	0·121	A few small exudates at the periphery, gradually increasing in number
	(4) 5h.18m. „ „	0·098	
3	(1) Before dose	0·117	During the day retinal exudates increased in size and number
	(2) 7h. after „	0·099	
4	(1) Before dose	0·107	Increase in exudates, fine lens striae
	(2) 6h.10m. after „	0·091	

TABLE IV B.—Rabbit VI (a) Control on glucose.

Days	Time of bleeding	Blood-sugar	Ocular lesions
1	(1) Before dose	0·091 gm. %	None
	(2) 40m. after „	0·117	
	(3) 1h.40m. „ „	0·114	
	(4) 2h.2m. „ „	0·114	
	(5) 3h.14m. „ „	0·109	
	(6) 8h.30m. „ „	0·098	
2	(1) Before dose	0·101	None
	(2) 42m. after „	0·122	
	(3) 3h.17m. „ „	0·108	
	(4) 6h.30m. „ „	0·107	
3	(1) Before dose	0·107	None
	(2) 7h.13m. after „	0·095	
4	(1) Before dose	0·108	None
	(2) 6h. after „	0·093	

(b) On naphthalene.

Days	Time of bleeding	Blood-sugar	Ocular lesions
1	(1) Before dose (2) 50m. after .. (3) 2h.30m. (4) 7h.	0.095 gm. % 0.096 0.096 0.095	Fundus clear
2	(1) Before dose (2) 7h. after ..	0.109 0.105	Minute retinal exudates Exudates larger
3	(1) Before dose (2) 6h.30m. after ..	0.095 0.098	Retina covered with large globular exudates
4	(1) Before dose	0.095	Confluent exudates

TABLE IV C.—Rabbit X, on naphthalene and glucose.

Days	Time of bleeding	Blood-sugar	Ocular lesions
1	(1) Before dose (2) 39m. after .. (3) 1h.14m. (4) 1h.57m. (5) 3h. (6) 7h.19m. (7) 9h.32m.	0.092 gm. % 0.178 0.149 0.124 0.100 0.094 0.110	Fundus clear Numerous small exudates at periphery of retina Exudates rapidly becoming confluent
2	(1) Before dose (2) 48m. after .. (3) 2h.37m. (4) 5h.10m.	0.099 0.171 0.115 0.095	Extensive retinal exudates early lens striae
3	(1) Before dose (2) 6h.39m.	0.094 0.101	Retinal and lens changes more advanced
4	(1) Before dose (2) 6h.35m. after ..	0.107 0.112	Opacity in posterior cortex both lenses, fundi not visible

Results. The following facts may be seen from the tables:—

(1) The "fasting" blood-sugar values showed only small variations. There was no marked hyperglycaemia (*cf.* Michail and Vancea), but in one case (Rabbit X), a slight rise occurred on the fifth and seventh days.

(2) In two rabbits which received naphthalene only, the blood-sugar showed an immediate slight fall in one (Rabbit XI), and remained practically constant in the other (Rabbit VIb). In

Rabbit X, on naphthalene and glucose, there was a rapid rise and fall in blood-sugar as might be expected, while the control (Rabbit VIa), showed a similar variation.

(3) The development of lesions in the retina or lens did not appear to coincide with any marked variation in blood-sugar.

In addition to these experiments it was found that rabbits on prolonged naphthalene dosage did not develop glycosuria, a symptom which one would expect if hyperglycaemia has also occurred. Further, the administration of a massive dose, *e.g.*, 50 gm., of glucose, by mouth (without naphthalene), did not affect the eyes, although on being repeated on several successive days it finally proved fatal.

Conclusion. There is no obvious correlation between the nature of the intra-ocular changes and the type of variation which occurs in the blood-sugar after naphthalene.

2. Variation in Blood Cholesterol

Historical. Michail and Vancea (1926) have reported that a marked hypercholesterinaemia occurred in two rabbits after repeated doses of naphthalene. A daily dose of 1.0 gm. was given in glycerine, and the dose was increased by 0.5 gm. each week. One animal which developed retinal lesions on the 13th day, and cataract on the 23rd day, showed a rise in blood cholesterol from 0.50 to 1.45 gm. per 1,000 c.c. The other which developed retinal lesions only on the 22nd day, showed a rise from 0.70 to 1.35 gm. per 1,000 c.c. with a continued rise to 1.50 after the cessation of the dose, and without the subsequent development of cataract. The authors regarded this change as a defensive reaction to a toxic substance, and compared it with their estimations of blood cholesterol in cases of human cataract, viz:—

Senile cataract—2.10 - 2.45 gm. per 1,000 c.c.

Congenital and juvenile cataract.—1.35 - 1.80 gm. per 1,000 c.c.

Experimental. Some further estimations of blood cholesterol were made, using the method of Myers and Wardell (1918), in order to see whether the same degree of hypercholesterinaemia occurred with a different type of dosage, and whether it showed any correlation with the appearance of the ocular lesions.

Naphthalene was administered in paraffin, and duplicate samples of blood were taken in the manner described for the blood-sugar estimations. In addition, one rabbit (A) was given repeated small doses of naphthalene in gelatin capsules until it developed deposits of crystals in its retinae (see Table I). The results are shown in the following table:

TABLE I.

Rabbit A.—On repeated small doses of naphthalene.

Rabbit B.—Control.

Rabbit A.			Rabbit B.	
Days	Naphthalene	Blood cholesterol	Days	Blood cholesterol
1	0.5 gm.	0.095 gm. %	1	0.092 gm. %
3	0.5	0.121	3	0.088
7	—	0.116	7	0.108
8	0.5	—		
9	—	0.119	9	0.085
10	1.0	0.112		
13	—	0.110	14	0.092
20	1.0	—	20	0.088
21	—	0.115		
22	1.0	—		
24	1.0	—		
27	1.0	—	27	0.100
30	1.0	—		
31	1.0	—		
34	1.0	0.118		
36	1.0	—		
38	1.0	—		
41	1.0	—		
43	1.0	—	44	0.092
45	1.0	—		
48	1.0	—		
49	1.0	—		
50	1.0	0.105	50	0.089

N.B.—Rabbit A developed crystals on the 45th day which were seen ophthalmoscopically and were afterwards identified in sections of the retinae. The animal maintained an average weight of 4 kgm. during the experiment.

TABLE II.

Showing immediate Variation in Blood Cholesterol.

Rabbit XIII, on 2 gm. naphthalene and 3 gm. glucose daily.

Days	Time of bleeding	Blood cholesterol	Ocular lesions
1	(1) Before dose (2) 40m after .. (3) 3h.7m. (4) 7h.	0.100 gm. % 0.108 0.101 0.101	Fundus clear
2	(1) Before dose (2) 7½h. after ..	0.115 0.109	No exudates visible Numerous small exudates
3	(1) Before dose	0.093	Extensive retinal exudates

The animal was killed by misadventure in dosing on the third day.

TABLE III.

Showing daily Variation in Blood Cholesterol.

(a) Rabbit VI, on 2 gm. naphthalene daily, followed by immediate access to food.

Days	Time of bleeding	Blood cholesterol	Ocular lesions
1	(1) 10.55 a.m. Before dose (2) 50m. after dose (3) 7h. " "	0.0680 gm. % 0.0681 0.0721	Fundus clear
2	10.0 a.m. before dose	0.0759	Small exudates at 10 a.m.
3	11.0	0.0715	Increase in number of exudates
4	11.0	0.0786	Increase in size of exudates
7	10.10	0.0866	Large confluent exudates
8	10.30	0.0760	Large confluent exudates
10	10.0	0.0766	A few crystals in both retinae
14	10.45	0.0728	Slight lens opacities, increase
17	2.45	0.0792	in number of crystals

TABLE III.—continued.

(b) Rabbit XII (a) As control on 3 gm. glucose and paraffin daily.
(b) On 3 gm. glucose and 2 gm. naphthalene daily.

Days	Time of bleeding	Blood cholesterol	Ocular lesions
(a) 1	(1) 10.55 a.m. before dose (2) 3.15 after dose	0.070 gm. % 0.068	Fundus clear
2	10.40 after dose	0.076	
3	11.20	0.070	
7	10.30	0.074	
(b) 8	4.15	0.068	Fundus clear
9	2.15	0.081	Fine lens striae, and small exudates
10	10.20	0.093	Changes advancing
14	10.50	0.073	Lenticular striae, but no opacity. A few crystals in retinae
17	2.40	0.078	

Results. At the time of appearance of retinal exudates a slight rise in blood cholesterol occurred in Rabbits XII and VI, but not in XIII. An initial rise occurred also in Rabbit A in the absence of any retinal changes.

Later, *i.e.*, in about 5—7 days, the blood cholesterol fell to a lower value, and remained stationary at a time when crystals were being deposited in the eye.

Conclusions. It appears that naphthalene gives rise to a slight degree of hypercholesterinaemia during the first week of administration. Unfortunately, the animals used only developed partial

lens opacities, but the figures obtained did not suggest that a marked rise in blood cholesterol would occur with the development of complete cataract. In view of this fact, and of the tedious nature of the estimations, no further experiments were done. It is impossible at this stage to attach any significance to the initial rise in blood cholesterol.

It is interesting to note that although in Rabbits VI, XII and XIII, the dose of naphthalene was much larger than that employed by Michail and Vancea, the rise in blood cholesterol was less, while the retinal changes developed much more rapidly.

3.—Variation in Blood Calcium

Historical. There are no published figures showing the changes in blood calcium after the administration of naphthalene. Michail and Vancea have stated, however (1927), that it remains constant except in acute naphthalene poisoning, when a pronounced fall occurs in the 24 hours preceding the death of the animal. Cade and Barral (1928), investigated the calcium content of various body-tissues, including blood, of rabbits with ocular lesions due to naphthalene. Their results are indefinite but suggest that the calcium content is raised above normal in some tissues.

Experimental. A series of estimations were made using the same procedure of dosage, etc., as described formerly.

Calcium was estimated in the serum rather than in the plasma, although it has recently been shown by Stewart and Percival (1928), that plasma contains 10—20 per cent. more calcium than the corresponding serum. In obtaining repeated samples of blood from a rabbit by bleeding from the marginal ear-vein, it is easier, and in some ways more satisfactory, to allow the blood to clot and to use the serum, rather than to attempt the elaborate precautions required for the collection of plasma. Clark and Collip's modification of the method of Kramer and Tisdall (1925) for estimating calcium in serum, is satisfactory for comparative determinations, if certain precautions are taken. All samples should be treated in the same manner, *e.g.*, in the time allowed for clotting, in the concentration of ammonium oxalate and in the time allowed for precipitation of the calcium as oxalate. The latter was never less than three hours and preferably the samples were left to precipitate overnight.

In order to obtain enough serum for duplicate estimations 6—8 c.c. of blood were required. Although this is a relatively large amount to take repeatedly, control experiments showed that the calcium did not alter by more than 1 mgm. as a result of bleeding.

A large series of rabbits were used, which developed all types of lesions due to naphthalene. A good deal of attention was paid to

the variations in calcium which accompanied the retinal exudates and the deposition of crystals in the retina, as they were found to be most interesting. So far, it has not been possible to follow the variation as far as the development of complete cataract. Some typical examples from the experiments are recorded in the following tables, showing the variation in serum-calcium in retinal exudation after :—

- I single dose of naphthalene,
- II naphthalene and glucose,
- together with control experiments on the effects of,
- IIIa paraffin alone,
- IIIb an inadequate dose of naphthalene,
- IV naphthalene and calcium gluconate,
- V calcium gluconate alone.

Examples are also given of the variation in serum calcium during the process of deposition of crystals after,

- VI repeated small doses of naphthalene
- VII naphthalene and glucose
- VIII one dose of naphthalene.

TABLE I.
*Variation in Serum Calcium in Retinal Exudation after a
Single Dose of Naphthalene in Paraffin.*

Rabbit	Weight	Dose	Time of bleeding	Serum calcium	Ocular lesions
XXXI	2.1 kg.	3.0gm.	(1) 19h. before dose (2) 45m. " " (3) 1h.40m. after " (4) 3h.45m. " " (5) 6h.30m. " " (6) 22h.35m. " "	15.39mgm. % 15.63 15.03 14.67 13.17 12.10	Vitreous haze in 3½ hrs., extensive exudates in 6½ hrs. Marked lens opacity on 6th day
31	1.6 kg.	3.0gm.	(1) 5m. before dose (2) 2h.40m. after " (3) 5h.5m. " " (4) 6h.25m. " " (5) 22h.30m. " "	14.67 12.49 12.18 11.83 11.59	Retinal haze in 6½ hrs., large confluent exu- dates seen after 22½ hrs.
XXX	2.1 kg.	3.0gm.	(1) 19h. before dose (2) 20m. " " (3) 1h.30m. after " (4) 3h.30m. " " (5) 6h.50m. " " (6) 23h.10m. " "	14.45 14.90 13.80 14.20 13.98 11.70	Fundus clear for 7 hrs. Confluent exudates at periphery, globular ex- udates at centre, seen after 23 hrs. Early lens striae also present
30	1.6 kg.	2.5gm.	(1) 15m. before dose (2) 3h.40m. after " (3) 5h.5m. " " (4) 7h. " " (5) 24h. " "	12.12 12.08 12.03 10.91 10.13	Fundus clear for 7 hrs. A number of small ex- udates seen in 24 hrs.

N.B.—Animals XXXI, 31 and XXX remained healthy. XXXI was killed one month later when it had atrophic retinæ, degeneration of the choroid, and incomplete cataracts.

Rabbit 30 developed severe diarrhoea on the second day and died on the third day.

TABLE Ia.—*continued.*

Rabbit LI, weight 2.25 kgm. Given 3 doses of 3 gm. naphthalene.

Days	Time	Serum calcium	Ocular lesions
1	5.15 D.5.30	15.26mgm. %	Fundus clear
2	9.30 5.15	13.50 13.30	General retinal haze, early lens striae. No further development
3	3.15 D.5.0.	15.82	
4	5.20 D.5.45	12.98	
5	12.15	10.57	

NOTE.—This animal showed a fall after the first dose, followed by a recovery as no dose was given on the second day. There was another fall on repetition of the dose, with general oedema of the retina.

TABLE II.

Variation in Serum Calcium in Retinal Exudation due to Naphthalene and Glucose.

Rabbit	Days	Time	Serum calcium	Ocular lesions
XIV	1	12.20 p.m. D.2.50	15.64 mgm. %	Fundus clear
	2	10.30 D.11.20 3.40	12.25 11.68	Extensive exudates
	3	3.20 D.3.30	11.58	
XVI	1	2.45 p.m. D.3.45	N.B.—Animal 15.34 mgm. %	died on the 4th day Fundus clear
	2	12.0 D.1.50 D.2.0 5.45	11.37 10.63 10.66	Retinal haze Minute peripheral exudates, and lens striae Exudates steadily increased in size
			N.B.—Animal	died on the 5th day

NOTE.—Each dose (D), consisted of 3.0 gm. glucose and 3.0 gm. naphthalene.

TABLE III.

Control experiments.

(a) Variation in Serum Calcium after Paraffin alone.

Rabbit	Weight	Dose	Time of bleeding	Serum calcium	Ocular lesions
28	1'6 kg.	15 c.c.	(1) 35m. before dose (2) 1h.55m. after „ (3) 4h.10m. „ „ (4) 6h.20m. „ „	11'16 mgm.% 11'71 11'68 11'48	None
37	3'0 kg.	15 c.c.	(1) 35m. before dose (2) 1h.30m. after „ (3) 4h.5m. „ „ (4) 6h.50m. „ „ (5) 23h.45m. „ „	12'79 12'59 12'25 12'75 12'76	None

(b) Variation in Serum Calcium after an inadequate dose of Naphthalene in Paraffin.

Rabbit	Weight	Dose	Time of bleeding	Serum calcium	Ocular lesions
38	3'1 kg.	1'5 gm. in 15 c.c.	(1) 25m. before dose (2) 1h.40m. after „ (3) 4h.20m. „ „ (4) 7hr. „ „ (5) 25h.15m. „ „	11'92 mgm % 12'20 11'44 12'00 11'82	None
39	2'7 kg.	1'0 gm. in 15 c.c.	(1) 10m. before dose (2) 1h.45m. after „ (3) 4h.35m. „ „ (4) 7h.10m. „ „ (5) 25h.30m. „ „	10'16 10'25 10'83 9'50 10'00	None

TABLE IV.

*Variation in Serum Calcium after Naphthalene and Calcium Gluconate.**(a) Rabbit 43, weight 2'5 kgm. Given 3'0 gm. naphthalene per os, and 1'0 gm. calcium gluconate subcutaneously, 2h. 30m. later.*

Dose	Time of bleeding	Serum calcium	Ocular lesions
Naphthalene	(1) 10.35 a.m. at 10.40	13'60 mgm.%	None
Calcium gluconate	(2) 12.25 p.m. at 1.10	13'51	
	(3) 2.25	14'26	
	(4) 4.10	12'60	
	(5) 5.10	13'55	None after 24 hours

(b) Rabbit 42, weight 2.5 kgm. Given 3.0 gm. naphthalene *per os*, and 1.0 gm. calcium gluconate subcutaneously, at the same time.

Dose	Time of bleeding	Serum calcium	Ocular lesions
Naphthalene and calcium gluconate	(1) 10.25 a.m. at 10.45	13.05 mgm. %	Fundus clear
	(2) 12.15 p.m.	13.57	
	(3) 2.0	13.72	
	(4) 4.5	13.62	
	(5) 5.15	10.65	Small retinal exudates
	(6) 11.10 a.m.	10.16	Large confluent exudates

N.B.—The animal remained healthy.

TABLE V.

Control experiments.

Variation in Serum Calcium after a subcutaneous injection of Calcium Gluconate.

Rabbit	Weight	Dose	Time of bleeding	Serum calcium	Ocular lesions
34	2.2 kg.	1.0 gm. in 15 c.c. H ₂ O	(1) 5m. before dose (2) 1h. after „ (3) 2h.35m. „ „ (4) 4h.55m. „ „	9.24 mgm. % 12.75 12.99 10.65	None
35	1.2 kg.	1.5 gm. in 15 c.c. H ₂ O	(1) 15m. before dose (2) 2h. after „ (3) 3h.30m. „ „ (4) 6h. „ „	13.69 17.05 14.12 13.07	None

TABLE VI.

Variation in Serum Calcium in deposition of crystals after slow dosage with Naphthalene.

Rabbit XVII, weight 1.75 kgm.

Days	Naphthalene	Serum calcium	Ocular lesions
1	0.5 gm.	16.78 mgm. %	Fundus clear
2	1.0	16.36	
3	1.0	—	
5	1.0	16.10	
7	1.0	15.96	
9	2.0	—	
12	3.0	16.05	
13	3.0	—	
14	3.0	16.16	
15	3.0	—	
16	3.0	16.05	A few crystals in retinae, increasing each day in number.
17	1.0	—	
19	3.0	16.62	Lenses clear

N.B.—The serum calcium was estimated each day before the animal was fed or dosed.

TABLE VII.

Variation in Serum Calcium in deposition of crystals after Naphthalene and Glucose.

(a) Rabbit XV, weight 3·4 kgm. (a) undosed, (b) on 3 gm. naphthalene, 3 gm. glucose daily.

Days	Time	Serum calcium	Ocular lesions
(a) 1	12.30	14·75 mgm. %	Fundus clear
2	10.45	14·90	
	4.0	14·60	
3	2.50	14·00	
5	11.30	14·53	
(b) 8	11.40	15·23	Fundus clear
	D. 3.0		
	4.45	15·36	
9	2.15	15·96	
	D. 3.0		
	5.30	15·90	
10	2.35	14·94	
	D. 4.0		
	D. = dosed		Crystals on 11th day.

TABLE VII.—continued.

(b) Rabbit XXXII, weight 2·6 kgm. (a) dosed with paraffin; (b) after one dose 3·5 gm. naphthalene in paraffin and 3 gm. glucose.

Days	Time	Serum calcium	Ocular lesions
(a) 1	10.35	15·19 mgm. %	Fundus clear
	2.00	15·21	
2	10.40	15·57	
4	9.45	15·29	
	5.00	14·86	
5	3.45	14·83	
(b) 6	D. 10.15		Fundus clear
	5.30	15·00	
7	—	—	A few minute exudates and lens striae
8	9.45	15·71	Crystals in retinae

TABLE VIII.

Variation in Serum Calcium in deposition of crystals after a single dose of Naphthalene.

Rabbit 29, weight 1.6 kgm. Given 2.5 gm. naphthalene in 15 c.c. paraffin.

Time of bleeding	Serum calcium	Ocular lesions
(1) 20 mins. before dose	11.45 mgm. %	Fundus clear
(2) 2 hrs. 10 mins. after dose	12.64	
(3) 4 hrs. 20 mins. „ „	13.91	
(4) 6 hrs. 50 mins. „ „	13.42	
(5) 24 hrs. „ „	11.37	
		Crystals seen in the retina on the 3rd day

Results. (1) The development of retinal exudates, whether they are caused by naphthalene, or by naphthalene and glucose, is accompanied by a marked fall in serum calcium. (See Table I, Ia, II and Fig I). The fall begins at a variable time after the dose.

(2) In comparison, the variation after a dose of naphthalene too small to cause a lesion, or after paraffin alone, is very slight, *i.e.*, usually less than 1 mgm. (See Tables III *a* and *b* and Fig. II).

(3) The process of deposition of crystals in the retina seems to be accompanied by the maintenance of a steady serum calcium of normal value. (See Tables VI, VII and VIII).

(4) A preliminary attempt was made to ascertain the effect of the administration of calcium gluconate together with naphthalene, in an amount calculated to neutralize the fall in calcium produced by the naphthalene. When the two substances were given together, the calcium gluconate did not prevent the retinal lesions nor a subsequent fall in serum calcium (see Table IV *b*). But on giving calcium gluconate two and a half hours after the naphthalene the fundus remained clear, and there was little change in the serum calcium (see Table IV *a*). In neither case was there a rise in serum calcium which was at all comparable with that produced by calcium gluconate alone (see Table V and Fig. III). It is hoped to repeat this experiment at a later date, on a larger number of animals).

Conclusion. The process of retinal exudation is accompanied by a definite disturbance in the blood serum calcium. The significance of this can only be made clear by further experiments, but in view of the fact that a similar change occurs also in retinal exudation due to quinoline (see Section II), it is probably of some pathological importance.

FIG. I. Fall in serum calcium in retinal exudation after naphthalene (see Table I).

FIG. II. Control experiments showing slight variation in serum calcium after (a) paraffin alone (28 and 37). (b) An inadequate dose of naphthalene (38 and 39) (See Tables III A and III B)

FIG. III.. The variation in serum calcium after (a) calcium gluconate and naphthalene (42 and 43). (b) Calcium gluconate alone (34 and 35) (See Tables IV and V)

Section II

On the Metabolism of Naphthalene and the Action of its Derivatives

Although naphthalene, being a double-ring compound is chemically a very stable substance, one would not expect it to be absorbed into the body unchanged. In agreement with this view, there is evidence that naphthalene itself has no direct action on the lens. Ovio and Manca (1896—8) and Salfner (1904), agree that animal lenses will remain quite clear after prolonged immersion in an emulsion of naphthalene. Ovio and Manca also found that the introduction of naphthalene into the conjunctival sac of a frog had no effect on the lens, while Hess (1887) and Salfner obtained negative results from the injection of naphthalene in olive oil into the anterior chamber. Klingmann (1897) reports the onset of irido-cyclitis and lens opacities after a similar experiment, but the ease with which sepsis might follow such an injection, is the probable explanation of these changes.

(a) **The effects of Dosage with Substances other than Naphthalene**

Several observers have stated that commercial naphthalene is more certain to produce ocular changes than is the pure substance, thus suggesting that the effects might be due to some impurity. Certain derivatives of naphthalene have been used in dosing, in the hope of elucidating the metabolism of the latter substance. Thus Kolinski (1889) tried nitronaphthalene, β -naphthol and anthracene, and Salfner tried chloronaphthalene, naphthalene dichloride, naphthalene sulphonic acids (α and β) and naphthaquinone without producing any ocular changes in rabbits. Salfner obtained a doubtful acceleration in the onset of lens changes by giving phthalic acid four or five days before a comparatively small dose of naphthalene.

van der Hoeve reports the appearance of retinal exudates, and of slight lens lesions in eight days after a dose of β -naphthol, a substance which has been known to cause retinal lesions in man. (Salfner 1904, van der Hoeve 1907). Igersheimer and Ruben (1910) deny that β -naphthol will cause lens lesions, and state that in large doses it is very toxic. They also obtained negative results from α -naphthol, although in a few cases of naphthalene poisoning they detected α -naphthol in the serum, aqueous humour and vitreous. Takamura (1911) injected a solution of α -naphthol intravenously into a rabbit, and found that small yellow spots appeared on the retina. They did not, however, enlarge or become confluent after the manner of exudates due to naphthalene, and seemed to correspond histologically to vacuoles in the outer nuclear layer. There were no lens lesions. According to Kuwabara (1911), lenses exposed to 1/100 solutions of α or β naphthalene sulphonic acids developed a definite opacity which he ascribed to the deleterious action of organic acids on the lens.

Jess (1921) made the interesting observation that quinoline tartrate administered to a rabbit intravenously, by mouth or subcutaneously, in doses of 0.1–0.2 gm. per kgm. body weight caused white spots in the retina which closely resembled the exudates due to naphthalene. Further, with small repeated doses a deposition of crystals in the retina occurred, and in a few cases, slight lens opacities also appeared. In addition, both types of retinal change showed a close resemblance histologically to those due to naphthalene. Jess tried benzene and pyridine without result, and found that acridine, tryptaflavin and argo-flavin will form slight retinal lesions which can only be detected microscopically. Optochin, which contains a quinoline nucleus, caused no lesions in rabbits though apparently Schanz (1913) was able to produce them by intravenous injection of this substance.

Experimental. My own experiments may be recorded as follows:—

(1) Several derivatives of naphthalene were administered to rabbits in order to see whether they would cause ocular lesions. The following substances gave negative results when given by mouth in aqueous suspension—*a*-naphthylamine hydrochloride, *β*-naphthylamine hydrochloride, naphthalene-1-sulphonic acid and naphthalene-2-sulphonic acid, each being tried in doses up to 3.0 gm. in rabbits of about 1.7 kgm. weight. *a*-Naphthylamine hydrochloride and the 1-sulphonic acid were toxic and caused death after a dose of 3.0 gm. *a*-Naphthol and *β*-naphthol given by mouth in suspension of liquid paraffin in doses up to 2.0 gm. also seemed to be entirely innocuous (*cf.* Bouchard, 1887, found the lethal dose of *β*-naphthol to be 3.8 gm. when given per os to a rabbit).

Some interesting results were obtained with quinoline tartrate. In confirmation of Jess's observation, doses of 0.2—0.3 gm. per kgm. body-weight gave rise to retinal lesions very like those due to naphthalene, but they remained in the form of discrete exudates and did not become confluent. The substance was administered subcutaneously, and in several cases the animal became collapsed immediately after the injection, with a very rapid heart-rate and respiration, but recovered entirely in about two hours. *An estimation of the serum calcium showed that the development of retinal exudates was accompanied by a fall in serum calcium very like that occurring after naphthalene*, which has been described in the previous section. The accompanying tables are a summary of the results obtained with quinoline.

TABLE I.
The Variation in Serum Calcium after inadequate doses of Quinoline Tartrate.

Rabbit	Weight	Dose	Time of bleeding	Serum calcium	Ocular lesion
13	kgm. 2.4	0.5 gm. in 20c.c. water per os	(1) before dose (2) 3h.30m. after .. (3) 5h.30m.	mgm.% 11.33 12.06 12.41	None
15	2.3	0.7 gm. in 30c.c. water per os	(1) before dose (2) 2h.30. after .. (3) 5h.5m.	13.65 13.01 12.13	None
15a	2.3	0.9 gm. in 30c.c. water per os	(1) before dose (2) 1h.40m. after .. (3) 4h.35m. (4) 6h.50m.	11.35 11.72 11.86 10.88	None

TABLE I—*continued.*

Rabbit	Weight	Dose	Time of bleeding	Serum calcium	Ocular lesion
26a	1·65	0·6 gm. in 15c.c. water <i>subcut.</i>	(1) 50m. before dose (2) 2h.20m. after „ (3) 5h.20m. „ „ (4) 7h.20m. „ „	12·03 12·36 12·36 11·72	None
27a	1·9	0·7 gm. in 15c.c. water <i>subcut.</i>	(1) 5m. before dose (2) 2h.10m. after „ (3) 5h.50m. „ „ (4) 7h.45m. „ „	11·88 12·25 11·08 10·72	None

N.B.—All these animals remained healthy, except 27a which died three days later from pneumonia. Both 26a and 27a showed collapse for 1½ hours after injection, but recovered in a few hours.

TABLE II.

The Variation in Serum Calcium after doses of Quinoline causing only slight retinal lesions.

Rabbit	Weight	Dose	Time of bleeding	Serum calcium	Lesions
13a	kgm. 2·25	1·0 gm. in 30c.c. water <i>per os</i>	(1) 1h. before dose (2) 5h.6m. after „ (3) 7h.15m. „ „	mgm. % 11·00 11·49 11·46	A very few exudates 24 hrs. later
16	2·8	0·8 gm. in 30c.c. water <i>per os</i>	(1) before dose (2) 2h. after „ (3) 4h.30m. „ „	13·38 13·24 12·57	A very few small exu- dates
16 (2 days later)	2·8	1·0 gm. in 30c.c. water <i>per os</i>	(1) before dose (2) 2h.5m. after „ (3) 4h.50m. „ „ (4) 7h. „ „	12·18 11·57 11·86 11·89	No further exudates
16 (1 week later)	2·7	0·8 gm. in 25c.c. water <i>subcut.</i>	(1) before dose (2) 3h.30m. after „ (Further samples lost)	12·54 10·81	Fresh exu- dates seen in 3½ hrs., increased rapidly in number
14a	2·3	0·7 gm. in 28c.c. water <i>per os</i>	(1) 45m. before dose (2) 2h. after „ (3) 3h.45m. „ „ (4) 5h.50m. „ „	10·26 10·32 9·12 9·67	A few old exu- dates present from pre- vious dose of 0·7 gm. No fresh exu- dates de- veloped

N.B.—All these animals remained healthy.
The third experiment on Rabbit 16 was inserted for comparison.

TABLE III.

The Variation in Serum Calcium after doses of Quinoline causing retinal lesions.

Rabbit	Weight	Dose	Time of bleeding	Serum calcium	Ocular lesions
14	kg. 2.3	0.7 gm. in 28c.c. water <i>per os</i>	(1) before dose (2) 2h.30m. after „ (3) 5h.30m. „ „	mgm. % 13.14 12.07 10.54	Numerous ex- udates in 4h. after dose
23	2.2	0.8 gm. in 16c.c. water <i>subcut.</i>	(1) 1h. before dose (2) 2h.30m. after „ (3) 4h.10m. „ „ (4) 5h.50m. „ „	14.75 15.09 15.01 12.15	A small num- ber of exudates after 5½h.
24	1.9	0.7 gm. in 16c.c. water <i>subcut.</i>	(1) 45m. before dose (2) 2h.40m. after „ (3) 4h.20m. „ „ (4) 6h. „ „	15.25 14.52 11.68 11.02	Numerous ex- udates in 2½ hrs. Condition progressed rapidly
32	2.65	1.0 gm. in 15c.c. water <i>subcut.</i>	(1) 25m. before dose (2) 5h.35m. after „	12.59 10.85	Numerous ex- udates in 3hrs. Many conflu- ent in 5hrs.
33	2.65	1.0 gm. in 15c.c. water <i>subcut.</i>	(1) 15m. before dose (2) 3h.45m. after „ (3) 6h.5m. „ „	13.18 12.66 11.44	Large number of discrete ex- udates in 3½ hrs.

N.B.—Animals 14 and 24 remained healthy subsequently, but 23 and 32 died two days later, and 33 died three days later. 24, 32 and 33 showed temporary collapse for about 1½ hours after the injection, but then recovered

TABLE IV.

Control experiments.

(a) *Variation in Serum Calcium after Water alone.*

Rabbit	Weight	Dose	Time of bleeding	Serum calcium	Ocular lesions
26	kg. 2.0	18 c.c. <i>subcut.</i>	(1) 20m. before dose (2) 2h. after „ (3) 4h.15m. „ „ (4) 7h.50m. „ „	mgm. % 13.60 13.95 14.13 14.27	None
27	1.85	30 c.c. <i>per os</i>	(1) 5m. before dose (2) 1h.55m. after „ (3) 4h.30m. „ „ (4) 7h.45m. „ „	13.01 12.88 13.05 13.50	None

(b) *Variation in Serum Calcium after Sodium Potassium Tartrate given subcutaneously.*

Rabbit	Weight	Dose	Time of bleeding	Serum calcium	Ocular lesions
29	kg. 2.0	0.40 gm. ($\approx$ 0.60 gm. quinoline tartrate) in 14 c.c. water	(1) 25m. before dose (2) 1h.10m. after " (3) 3h.30m. " " (4) 6h.55m. " "	mgm.% 10.95 10.97 10.45 11.02	None
36	3.1	0.80 gm. ($\approx$ 1.20 gm. quinoline tartrate) in 14 c.c. water	(1) 5m. before dose (2) 1h.40m after " (3) 4h.25m. " " (4) 7h. " " (5) 23h.20m. " "	12.31 12.70 12.93 12.15 11.82	None

Conclusion. It can be seen from the above tables that the development of retinal exudates is accompanied by a marked fall in serum calcium (Table III,) whereas doses of quinoline tartrate which cause no lesions or only very slight ones cause a fall of about 1 mgm. only (Tables I and II). In control experiments on the effect of a subcutaneous injection of water or a soluble tartrate, the variation in calcium was not more than 0.6 mgm. (Table IV).

(2) In addition, *substances which might alter the reaction of the gut*, and so affect the absorption of naphthalene, were tried alone, and with the latter substance. They were as follows:—

(a) Sodium bicarbonate, given with naphthalene in equal gram doses, did not prevent the appearance of retinal exudates.

(b) Calcium lactate (which also causes a definite rise in blood-calcium), given with naphthalene in equal gram doses, appeared to facilitate the deposition of crystals rather than the formation of exudates. In one rabbit which developed crystals the subsequent administration of naphthalene alone in large doses did not cause any exudates to appear. Calcium lactate alone even in large and repeated doses will not cause intra-ocular changes. (Adams, 1929).

(c) Sodium phosphate Na_3PO_4 , and dihydrogen sodium phosphate, given in aqueous solution by mouth in daily repeated doses up to 5.0 and 6.0 gm. respectively, caused no fundus changes.

(d) Glucose administered in equal gram doses with naphthalene always accelerated the appearance of retinal exudates and could be used as a sure means of producing them. As stated in Section I, glucose alone, even in very large doses, has no effect on the fundus.

FIG. 1. The variation in serum calcium after inadequate doses of quinoline tartarate (see Table I).

FIG. 2. The variation in serum calcium after doses of quinoline causing slight retinal lesions (see Table II).

FIG. 3. The fall in serum calcium after doses of quinoline causing numerous retinal exudates (see Table III).

FIG. 4. Control experiments showing slight variation in serum calcium after (a) water (26 and 27). (b) Sodium potassium tartarate (36 and 29). (See Tables IV A and IV B)

(3) Oxalates were also tried, because of the probability that the crystalline deposits in the retina are calcium oxalate. (*cf.* Adams, 1930).

(a) Oxalic acid.—Small repeated doses of oxalic acid, *e.g.*, beginning with 0.1 gm. and increasing by 0.1 gm. each day did not cause ocular lesions, but sections of the kidney showed numerous crystals in the tubules, which resembled the retinal crystals after naphthalene both in appearance and in being doubly-refractive. In larger doses oxalic acid caused death from severe gastro-enteritis.

(b) Sodium oxalate was found to be very toxic. A dose of 2.00 gm. by mouth caused violent gastro-enteritis in two hours, and death in 12 hours.

(c) Potassium oxalate in repeated doses up to the equivalent of 1.0 gm. of oxalic acid, for three days, did not cause fundus changes, but when administered in a small dose with naphthalene it seemed to increase the effects of the latter. Thus, in one rabbit 1.5 gm. naphthalene was without effect, but when this amount was given a week later with 0.78 gm. potassium oxalate large exudates and lens striae appeared within 24 hours. A second rabbit which received a single dose of 3 gm. naphthalene with 0.78 gm. potassium oxalate ($\equiv$ 0.5 oxalic acid), rapidly developed marked conjunctival oedema, retinal exudates, and complete cataract in a few days.

Conclusion. In the case of all substances administered with naphthalene it is difficult to design a real control experiment, but it would appear that glucose undoubtedly accelerates the effects of naphthalene, while potassium oxalate also does so but possibly in some different way. Calcium lactate probably favours the deposition of crystals by helping to maintain a normal blood calcium.

(b) The Excretory Products of Naphthalene

Several attempts have been made to trace the metabolism of naphthalene by means of colour tests on the serum and urine. Thus, Penzoldt (1886) and Lesnik (1888) described the development of a dark green colour on the addition of concentrated H_2SO_4 to naphthalene urine. According to Penzoldt this was due to the presence of β -naphthaquinone while Lesnik ascribed it to α -naphtholglycuronic acid and actually isolated the latter substance from human naphthalene urine.

Edlefsen (1904) described several tests and considered that in man, naphthalene was excreted chiefly in the form of β -naphtholglycuronic acid and β -naphthaquinone. The former substance was held responsible for the development of a cherry-red colour on the addition of a few drops of glacial acetic acid and of sodium nitrite to 8–10 c.c. of urine.

Igersheimer and Ruben (1919) obtained a positive diazo-reaction for a phenol in the serum and urine of a rabbit after naphthalene. From the spectroscopic absorption band they concluded that it was due to α -naphthol. The serum showed a positive reaction in 2—3 hours after a dose of 2 gm. naphthalene per kgm. body weight, while excretion began in three hours and continued for four days. Ocular lesions did not appear in these cases in which the serum gave a negative reaction, but Igersheimer and Ruben considered that some derivative of naphthalene, other than α -naphthol was the factor responsible for their development. Edlefsen's test was positive in the urine and occasionally in the serum also. They were unable to confirm Salfner's observation (1904) that blood taken from a naphthalinized rabbit would cause retinal exudates to appear within 12 hours of its injection into the ear-vein of a normal rabbit.

Lindberg (1921) also used the diazo-reaction to follow the course of naphthol into the foetus after the administration of naphthalene to a pregnant rabbit. After a relatively large dose of 5 gm. the serum was positive in two hours, gave a maximum reaction in 3—4 hours, and remained positive for 20 hours, while the reaction in the urine was very strong. The embryonic serum was positive if the mother's blood was also positive, while the amniotic fluid was positive in the latter days of pregnancy. The aqueous humour was positive in a few cases only in the third paracentesis.

Experimental. A certain amount of information concerning the metabolism of naphthalene in rabbits was obtained by repeating these and certain other colour tests and tracing their distribution in the serum and in differently-extracted portions of naphthalene urine.

Examination of naphthalene urine. By keeping rabbits in a metabolism cage the urine could be collected without contamination during the period of dosage with naphthalene. Soon after the latter was commenced, it was noticed that the upper layers of the urine became dark brown as though some kind of oxidation was taking place in the air. Later, the whole of the urine passed was brown. Tests for sugar, bile and blood (except in one severe case) were negative. Albumen usually appeared on the third or fourth day. Microscopically, large epithelial cells, but no tubular casts were seen, and red cells were very rarely present.

A large amount of urine was fractionated as follows:—

(1) It was tested unchanged, and then extracted repeatedly with ether till the ether extract was no longer yellow and gave a negative test with ferric chloride. (2) The ether extract was evaporated down to a small volume in a vacuum dessicator. (3) Part of the urine remaining after ether extraction was treated with an equal volume of water and distilled, while the rest was acidified

with HCl. (1 c.c. of 10-N.HCl. to each 50 c.c. of urine), hydrolysed for several hours, and then distilled. (4) The residue after hydrolysis and distillation was neutralized with Na_2CO_3 , tested and re-extracted with ether.

The results of analysis are shown in the table. The colour tests employed were the following:—

(1) *Diazo-test*. The reagent was prepared from sulphanilic acid after the manner described by Lindberg (1924). The test proved remarkably sensitive, being positive down to 0.05 mgm. of α -naphthol and to 0.1–0.2 mgm. of β -naphthol. It also enabled one to detect minute amounts of α -naphthol, e.g., 0.2 mgm. which had been added to normal serum.

TABLE I.
Analysis of "Naphthalene" Urine.

Fraction of Urine	Colour of Fraction	Tests						Other tests
		Diazo	Fe Cl ₃	Iodine and NaOH	Penzoldt	Edlefsen HAc	HCl	
1) Unchanged urine	Dark brown	+	+ mauve	+	+	+	+	(1) Glycuronic acid test negative (2) Red on boiling with HCl.
(2) Ether extract	Yellow-brown	+	+ mauve	+ mauve	—	—	—	
(3) Urine after ether extraction	Dark brown	+ weak	O	—	+	+	+	Glycuronic acid test negative
(4) Distillate from (3)	Clear, with a few colourless crystals	—	faint yellow-green ppt.	—	—	—	—	
(5) Distillate after acid hydrolysis of (3)	Pale yellow, with pink* crystals on evaporation	+ strong	+ green then mauve	+ mauve faded rapidly	—	—	—	
(6) Neutral residual urine	Reddish-brown	+	O	O	+	—	—	
(7) Ether extract of (6)	Yellow-brown	+	+ mauve	+ faded rapidly	—	—	—	(1) Benedict negative (2) Glycuronic acid test negative
(8) Residue from (7)	Reddish-brown	+ weak	green-brown ppt.	—	+ weak	—	—	(1) Glycuronic acid test positive (2) Benedict, reduced on standing (3) Green with Na_2CO_3

+, positive. —, negative. O, not done. *N.B.—Crystals resembled α -Naphthol.

The test was positive in rabbit urine within half-an-hour of a dose of naphthalene (of about 1 gm. per kgm. body weight). The corresponding serum was not always positive, *i.e.*, it was positive with doses of 3.5 gm. or more, but might be negative with smaller doses. It appeared in about two and a half hours, usually before the intra-ocular changes. The concentration in the serum was exceedingly small in comparison with that in the urine, in which it seemed to increase on standing.

(2) *Ferric chloride test.* An aqueous solution of ferric chloride is known to give a purple colour and precipitate with α -naphthol, and a green colour followed by a yellow-green precipitate with β -naphthol. It was found that the sensitivity of these reactions was as great as that of the diazo-reaction and they could therefore be used to confirm the latter. The solution to be tested was extracted with an equal volume of ether, the ether extract was evaporated to dryness on a water-bath, the residue was taken up in a little water and tested with ferric chloride.

(3) *Test with iodine and sodium hydroxide.* A solution of iodine in potassium iodide, together with an excess of sodium hydroxide, gives a cloudy violet colour with a solution of α -naphthol, while with β -naphthol the solution turns clear and colourless. This test was employed as an additional test for naphthols.

(4) *Penzoldt's test* was performed as a "ring" test by adding concentrated H_2SO_4 to urine.

(5) *Edlefsen's test.* As described previously, the addition of glacial acetic acid and of $NaNO_2$ to naphthalene urine, results in a cherry-red colour. According to Edlefsen, the use of HCl instead of acetic, results in a yellow colour, while with solutions of β -naphthol the reactions with the two acids are reversed. These tests were confirmed, and it was also found that α -naphthol is negative with both.

None of these tests excepting the diazo-test was positive in the serum after naphthalene, although several times an animal was bled to death under an anaesthetic in order to obtain large quantities of serum for testing.

In rabbits dosed with α - or β -naphthol, the diazo-test was positive in the urine after the first half-hour; but after doses up to 2.0 gm. by mouth, the serum was negative in every instance, although it was examined at frequent intervals.

Control tests on normal rabbit urine were negative throughout.

Conclusions. Although it is not justifiable to attempt to identify the end-products of the metabolism of naphthalene without isolating them from the urine, yet the tests described give a valuable indication as to what they may be.

The distribution of the diazo-, iodine and ferric chloride tests suggests that α -naphthol is present in the urine, and that a further amount is liberated by acid hydrolysis. The latter process liberates glycuronic acid and a certain amount of β -naphthol. It is, therefore, reasonable to suggest that in the rabbit as in other animals, naphthalene is excreted chiefly as α -naphthol, and as α - and β -naphtholglycuronic acids. Possibly one of the latter two substances is responsible for Edlefsen's test, as he himself suggested, since it is only positive in unhydrolysed urine. Penzoldt's test has been ascribed both to β -naphthaquinone and to α -naphtholglycuronic acid, but as it is still positive after prolonged hydrolysis, it is unlikely that it is due to either of these substances.

These metabolic products must be borne in mind when one attempts to attribute the pathological effects of naphthalene to causes of a more physiological type. Yet, so far, no chemical toxin has been found and apart from the diazo-test there has been no indication as to what derivative of naphthalene is in the circulation. By finding an occasional positive Edlefsen test in the serum, Igersheimer and Ruben suggested that α - or β -naphtholglycuronic acids were present there. In this connection it is interesting to note that β -naphtholglycuronic acid is known to form a soluble calcium salt which is easily soluble in water, and which would, therefore, provide a ready means of excretion.

Summary. The absence of any definite conclusion at this stage of the investigation is due to the necessity for following up so many different aspects of the problem. It is hoped that further work will bridge the gaps between them and will provide an adequate explanation of the action of naphthalene. There may be some significance in the fact that the development of retinal exudates is accompanied by a fall in serum-calcium whether they are due to naphthalene or to quinoline.

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