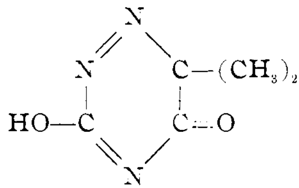
Calculated for one mol. N in one mol. triazine, 19.58 per cent. Found: N, 19.85.

In this connection it might be mentioned that carbonamidohydrazo-*i*-butyric acid, on treatment with bromine, evolved 17.47% N. Calculated for one mol. N in one mol. of acid, 17.39 per cent. This experiment makes it highly probable that carboamidoazo-*i*-butyric acid, $NH_2CON{=}NC(CH_3)_2COOH$, is also not capable of existence.

The preparation of 6-dimethyl-3-hydroxy-5-keto-1,2,4-triazine,



was attempted, because, from the formation of 6-methyl-3,5-dihydroxy-1,2,4-triazine from *α*-ureido-*α*-*m*-nitrophenylenediazoaminopropionic acid ethyl ester and alcoholic potash, it was to be expected that *α*-ureido-*α*-*m*-nitrophenylenediazoamino-*i*-butyric acid ethyl ester would show an analogous behavior. As has been described, the substance formed in this latter case is, however, in the main a potassium salt of the diazoamino body, minus alcohol. Two methods of preparation of the dihydroxy dimethytriazine in question suggested themselves from the work that one of the authors in connection with others has done on the corresponding methyltriazine[1]. First an attempt was made to oxidize with bromine water, 1,2-dihydro-6-dimethyl-3,5-dihydroxy-1,2,4-triazine. The experiments instituted in this connection are described above. In the second place an attempt was made to condense carbonamidoazo-*i*-butyric acid ethyl ester with sodium alcoholate to the desired triazine. This experiment was unsuccessful as the substances reacted violently with gas evolution, forming a colorless solution from which nothing was isolated.

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A PRELIMINARY COMMUNICATION ON THE TOXICITY OF SOME ANILINE DYESTUFFS.[2]

By Gustave M. Meyer.

Received April 20, 1907.

Introduction.

Recent discussions concerning the use of various artificial organic dyes as food colorants made it seem very desirable to study the toxic effects of

[1] loc. cit.

[2] Presented before the New York Section of the Am. Chem. Soc., Science, **25**, 343 (1907). See also, Proc. sect. Biol. Chem. of Am. Chem. Soc. with sect. C., A. A. A. S., Science, **25**, 457 (1907).

some of the pigments commonly used for such purposes. Previous investigations of this character consisted chiefly of observations of the general effects on rabbits and dogs (and in some instances on man) of various amounts of such substances, after their administration *per os* or subcutaneously. The results of such superficial studies have been considered sufficient to determine the possibility of using to commercial advantage particular pigments as food colorants.

The published conclusions of various investigators concerning the toxicity of a particular dyestuff were often contradictory. Thus, for example, Weyl[1] considered that orange II (sodium-β-naphthol-azobenzene sulphonate) was toxic. He found that a dog weighing 10.5 kg. succumbed on the 20th day of the experiment after three administrations per stomach tube of five, seven and two grams respectively (total, 14 grams) of this dye. Frentzel[2] having obtained contrary results after the administration of smaller doses disagreed with Weyl's general conclusion on toxicity. Chlopin's[3] experiments confirmed those of Weyl. Chlopin stated that he took 0.2 gram of the dye and felt very badly for about four hours, the evil effects gradually becoming less pronounced and disappearing about eight hours after taking it.

The above mentioned investigators reported similar contradictory results of experiments with metanil yellow (sodium-azodiphenylamino-*m*-azobenzenesulphonate). Whereas Weyl considered this substance toxic both Frentzel and Chlopin regarded it as non-toxic, at least in such amounts as are commonly used in foods. Chlopin took 0.2 gm. of metanil yellow without any ill effects.

Naturally, idiosyncrasy is shown toward certain dyestuffs[4] and experiments of the nature referred to on dogs and rabbits do not permit very positive conclusions regarding the effects of such substances on man. However, if exceptionally heavy doses can be withstood by dogs without any bad effects it may be presumed that such minute amounts as would commonly be consumed by an average person on an ordinary diet cannot be particularly harmful. That only small amounts of dyestuff are required for the coloration of foods is well known. The following, quoted from the translator's preface to Weyl's[5] book gives some facts in this connection :

"A manufacturing confectioner of this city, for whom I make examinations of colors used by him, informs me that a yellow color sold as auramine, has such high tinctorial power that one ounce will color 2,000 pounds of candy to the highest yellow tint required in his business. It

[1] Weyl : "The sanitary relations of the coal tar colors." Translated by Lippmann (1892).
[2] J. Frentzel. Z. Nahr.-Genussm., **4** ; 968 (1901).
[3] Chlopin : Ibid., **5**, 24 (1902). Hygienisches Rundschau, **13**, 201, and 253 (1903).
[4] Deut. med. Wochschr., 126 (1891).
[5] Weyl : loc. cit.

is obvious that the toxicity of such a body would have to be very high to render it harmful in such use.'' Conclusions of this kind do not take into account the possible detrimental action ensuing on healthy as well as diseased persons from long continued use of small quantities of foreign substances.

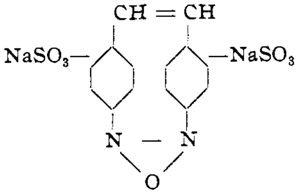
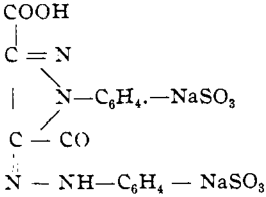
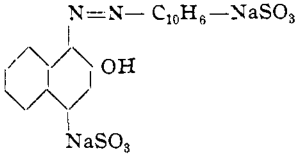
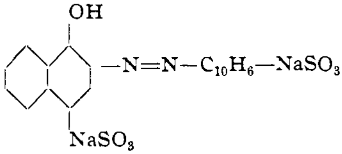
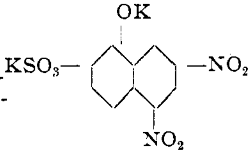
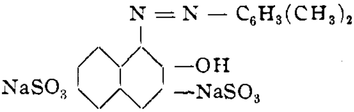
Obviously, any detrimental effects of a dyestuff depend to some extent on its influences on metabolism With a view of choosing a comparatively toxic dye for a study of this kind a number of commonly employed food colorants were studied as to their general behavior when given to dogs in varying amounts and during fairly long periods. A dyestuff may be toxic because of its inherent poisonous qualities or its toxicity may be due to the presence of some foreign toxic substance used in the manufacture of the dye, and not entirely eliminated in the process of preparation. It is of course essential that dyestuffs which are used as food colorants should not be prepared with the aid of reagents containing arsenic or any other such strongly poisonous material.

The colorants[1] used in my experiments together with their chemical nomenclature, constitution, and general application are listed in Table I. Commercial pigments are usually admixed with certain amounts of neutral colorless substances in order to render uniform the tinctorial powers of the dyestuffs sold under a particular trade name. The substances most commonly employed in this way are starch, glucose, cane sugar, sodium chloride and sodium sulphate. Sodium chloride may be present as an impurity because of its use in ''salting out'' the pigment.

Either the sulphate or the chloride of sodium has been found in the dyes used in these experiments. Table II gives the calculated amounts of ash theoretically obtainable from each colorant and also the corresponding amounts also estimated of ash found and as sulphate. The method used to obtain the ash was to ignite weighed amounts of the dye after drying at 110° to constant weight in a platinum crucible and then to convert all of the sodium to the sulphate by adding some concentrated sulphuric acid and heating to constant weight. In one column are given the figures for ash residues as obtained without the treatment with acid. These residues were not homogeneous but appeared to be mixtures of the sulphide, sulphite and sulphate of the particular alkali metal in the dye. As this investigation was entirely of a preliminary character, exact quantitative determinations in this connection were not attempted, especially as the dyestuffs were ordinary commercial preparations of the types commonly used for food coloration and which in themselves lack uniformity in the amount of contained fillers. The exact qualitative and quantitative determinations of the latter will be carried out in conjunction

[1] These colorants were samples of products put on the market in 1903 by H. Lieber & Co.

TABLE I.

Trade Name	Chemical Name	Constitutional formula[1]	Name indicating application
1. Curcumin S	Sodiumazoxy stilbendisulphonate		Egg color
2. Tartrazin	Di-sodium-1-p.-sulphoxyl phenyl 3-carboxyl 4-p-sulphoxyl phenyl hydrazone-5-pyrazolene		Macaroni color
3. Naphthol red S	Sodiumnaphth-azo-β-naphthol disulphonate		Tomato catsup color
4. Carmoisin B	(An isomer of Naphthol red S)		Raspberrry color
5. Naphthol yellow S	Potassium dinitro naphthol sulphonate		Mustard and pie filling color
6. Gold orange	Sodium dimethylaniline azobenzol sulphonate (Helianthin)	$(CH_3)_2N—C_6H_4—N=N—C_6H_4—NaSO_3$	Orange color
7. Ponceau 2 R	Sodium xylidine azo-2-naphtol 3-6-disulphonate		Strawberry red color

[1] The constitutional formulas are taken from the works of Schultz and Julius. 1902.

with the more detailed study of the effects of food colorants. The data presented in Table II indicate close approximations of the proportions in which the inorganic admixtures occurred :

TABLE II

	Filler	H_2O	Ash	Ash as Na_2SO_4	Calculated Na_2SO_4
		Per Cent.	Per Cent.	Per Cent.	Per Cent.
Curcumin S	Na_2SO_4	3.87	80.56	86.45	30.47
Tartrazin	NaCl	6.24	61.11	64.58	25.00
Naphthol red S	NaCl	4.96	67.36	86.10	28.28
Carmoisin B	NaCl	7.48	65.91	88.51	28.28
Naphthol yellow S	NaCl	1.08	47.29	62.37	43.03
Gold orange	Na_2SO_4	3.18	36.54	36.34	21.72
Ponceau 2 R	NaCl	9.26	55.19	65.16	29.58

Of the seven dyestuffs considered in this paper only naphthol yellow S and ponceau 2 R appear to have been investigated by others[1] from the standpoint of toxicity. The investigations reported in this paper include, besides observations of the general influence of these substances, also studies of their elimination in the urine, feces, bile and milk and their effects on peptic digestion in *vitro*.

Experimental

Dogs were used in these experiments. Each was kept in a suitable cage[2] throughout an experiment and fed once daily on a mixed diet of meat, cracker meal, lard and water in such proportions that the animals remained approximately constant in weight for some time during the predosage periods. After a preparatory period of reasonable length an amount of dyestuff equal to one-tenth gram per kilo of the weight of the animal was given in a meat ball before the main diet was offered. The animals were usually so eager for food that this procedure could often be dispensed with and the dyestuff directly mixed with the diet, without interfering with its ingestion.

The amount of dye used in each experiment was increased daily in geometric proportions until toxic symptoms were shown. The administration of dyestuff was then discontinued for a day or two to allow the animal to recuperate, after which the dog was again given such amounts as had previously been borne without marked disturbances. These conditions were maintained for several days after which the animals were chloroformed and subjected to post mortem examination. The autopsy included besides the usual general examination also an histological examination[3] of the liver, kidneys, stomach and intestines.

Experiment I.

Dog: male, weight 12.30 kgs. Diet: meat 225 gms., cracker meal 50

[1] Chlopin, also Weyl, loc. cit.

[2] Gies: Amer. J. Physiol., **14**, 403 (1905.)

[3] The histological examinations were carried out by Dr. E. R. Posner, whose cooperation in connection with a part of this investigation I heartily appreciated. I regret that his departure to Drake University deprived me of his further valuable assistance.

gms., lard 35 gms., bone ash 10 gms., and water 600 cc. After several days of feeding on this diet the dog's daily weight was constant at 12.80. kgs. Dyestuff: Curcumin S.

Days	Wt. of dog. kilos.	Curcumin. grams.	URINE Volume 24 hrs. c.c.	Sp. Gr.	Feces.
1.	12.80	1.28	340	1017	Normal
2.	13.00	2.57	420	1021	Normal
3.	12.96	5.14	440	1017	Normal
4.	13.15	10.28	500	1018	Diarrhea
5.	12.10	10.28	645	1027	Scant
6.	12.85	....	360	1031	Scant
7.	12.78	5.14	360	1020	Normal
8.	13.15	5.14	400	1028	Diarrhea
9.	13.00	5.14	460	1023	Normal
10.	12.89	5.14	550	1019	Normal
11.	13.02	5.14	540	1020	Normal
12.	12.94	5.14	610	1018	Slight diarrhea
13.	12.90	5.14	550	1020	Normal

The reaction of the urine was always acid and at no time could any reducing substance be detected. It was always orange colored after the first day. The fecal matter was also of an orange color during the period of dye administration. On the fourth day the dog was restless and had diarrheal movement in the afternoon, after which he became quiet. On the fifth day, one hour after feeding, the dog vomited. This may have been brought on by an effort to dislodge hair in his throat, of which he gave evidence in the usual manner. The vomitus consisted mainly of highly colored cracker meal. A small amount of undissolved coloring matter but no meat was discernible. The dog showed no further signs of illness.

Autopsy: Nothing abnormal could be detected and the histological examination gave no evidence of any alteration of the tissues. The tissues were not pigmented and no change of color was produced by treating them with either sulphuric acid or hydrogen peroxide or both.

Experiment II.

Dog: male, weight 9.60 kgs. Diet: meat 175 gms., cracker meal 40 gms., lard 30 gms., bone ash 10 gms., water 500 cc. Dyestuff: tartrazin.

Days	Wt. of dog. kilos.	Tartrazin grams.	URINE Volume 24 hrs. c.c.	Sp. Gr.	Feces.
1.	9.80	0.98	600	1015	Normal
2.	9.92	1.96	470	1013	Normal
3.	10.10	3.92	435	1013	Normal
4.	10.10	7.84	400	1014	Normal
5.	10.07	15.68	500	1017	None
6.	9.80	19.60	560	1015	Diarrhea
7.	9.48	....	818	1022	Soft
8.	9.82	....	152	1041	Scant
9.	10.10	15.68	165	1031	Soft
10.	10.10	15.68	540	1020	Soft
11.	10.10	15.68	445	1024	Soft
12.	10.10	15.68	265	1020	Soft
13.	10.10	15.68	380	1021	Soft

There were no special symptoms to be noted. The dog at no time showed signs of being ill. Diarrhea set in on the sixth day after a heavy dose of tartrazin had been given (19.6 gms.), although all the feces were colored by the pigment after the first administration. The color of the feces varied from a light yellow to a brownish red, the latter color persisting during the last five days of the experiment. The urine also had a decided yellow color, and always contained a trace of albumin (seminal?) but no reducing substance. The autopsy showed nothing abnormal. The bile contained tartrazin.

Experiment III.

Dog: female, weight 10.75 kgs. Diet: meat 190 gms., cracker meal 30 gms., lard 20 gms., bone ash 10 gms., water 500 cc. Dyestuff: naphthol red S.

Days	Wt. of dog kilos.	Naphthol red S. grams.	URINE Volume 24 hrs. cc.	URINE Sp. Gr.	Feces
1.	10.73	1.073	550	1013	Normal
2.	10.65	2.146	540	1013	Normal
3.	10.63	2.146	435	1012	Normal
4.	10.75	4.292	255	1029	Normal
5.	10.86	8.584	460	1018	None
6.	10.70	17.168	550	1020	None
7.	10.62	25.752	492	1030	Slight diarrhea
8.	10.65	....	815	1030	Diarrhea
9.	10.65	18.000	415	1025	None
10.	10.58	18.000	425	1027	Watery diarrhea
11.	10.33	18.000	670	1025	Normal
12.	....	18.000	710	1022	Normal
13.	10.10	18.000	348	1031	Normal
14.	....	20.000	...	....	......

The urine contained no albumin or reducing substance. It was always colored a deep red when fresh but lost this color on standing. The feces during the first eight days were red; toward the end of the experiment they were colored chocolate brown.

The autopsy showed in the main all parts to be normal. The stomach contents as well as the gastric mucosa were raspberry colored. The color of the gastric mucosa could not be washed off. The gall bladder was large and filled with a reddish colored bile. The subcutaneous areas were in general more pinkish than usual, even noticeable at the extremities. The connective tissues in the abdomen and also the tendons were pinkish. The color was accentuated on applying sulphuric acid; hydrogen peroxide caused a decoloration. The histological examination revealed nothing abnormal.

Experiment IV.

Dog: male, weight 12.20 kgs. Diet: meat 215 gms., cracker meal 50 gms., lard 35 gms., bone ash 15 gms., water 600 cc. Dyestuff: carmoisin B.

Days	Wt. of dog kilos.	Carmoisin B grams	URINE Volume 24 hrs. cc.	Sp. Gr.	Feces
1.	12.40	1.24	415	1013	Normal
2.	12.50	2.48	415	1012	Normal
3.	12.45	2.48	515	1012	Slight diarrhea
4.	12.50	4.96	450	1015	......
5.	12.51	9.92	390	1025	......
6.	12.50	19.84	475	1025	......
7.	12.50	19.84	358	1025	Strong diarrhea
8.	12.56	27.76	250	1032	.....
9.	12.30	....	547	1025	Soft
10.	12.67	15.00	375	1020	Normal
11.	12.55	15.00	410	1025	Normal
12.	....	15.00	230	1025	Normal
13.	12.90	22.50	360	1030	Normal
14.	19.00	19.00	...	....	......

With the exception of occasional diarrhea the dog showed no apparent ill effects. Diarrhea was marked only after the administration of exceptionally large doses of the colorant. The color of the stools was a deep violet and not at all like the original dyestuff. The freshly voided urine was carmine in color; on standing it soon turned dark.

The autopsy showed nothing abnormal. The gastric contents were colored red whereas the intestinal contents were dark brown changing gradually to a dark violet in the lower part of the gut. The membranes themselves were not stained, as the color could be readily washed off with water. The bile from the gall bladder was decidedly carmine in color.

Experiment V.

Dog: male, weight 18.77 kgs. Diet: meat 33 gms., cracker meal 73 gms., lard 55 gms., bone ash 20 gms., water 800 cc. Dyestuff: naphthol yellow S.

Days	Wt. of dog kilos	Naphthol yellow S grams.	URINE Volume 24 hrs. cc.	Sp. Gr.	Feces
1.	19.20	1.92	850	1012	......
2.	18.96	3.84	825	1012	Normal
3.	18.87	3.84	555	1013	Slight diarrhea
4.	18.90	3.84	635	1010	Slight diarrhea
5.	19.03	7.68	715	1010	Normal
6.	19.08	15.36	520	1017	......
7.	19.12	15.36	650	1020	......
8.	19.05	30.72	540	1024	Diarrhea
9.	18.80	....	745	1024	......
10.	19.00	10.00	585	1022	Normal
11.	19.13	10.00	595	1011	Normal
12.	....	10.00	590	1021	Normal
13.	19.00	10.00	537	1017	Normal
14.	....	10.00	...	....	......
15.	....	15.00	...	....	......

The stools were diarrheal after 3.84 gms. of naphthol yellow had been administered. Increasing amounts of the dye emphasized this tendency. The feces always had a dark brown color.

The urine was only slightly colored after administration of small doses of the pigment. Larger doses caused the urine to become a deep red. It was noticed that the urine in these cases when freshly voided was deep yellow which turned red on standing. Tests for albumin and reducing sugar were always negative.

The autopsy revealed nothing abnormal.

Experiment VI.

Dog: male, weight 6.3 kgs. Diet: meat 130 gms. cracker meal 25 gms., lard 20 gms., bone ash 5 gms., water 325 cc. Dyestuff: gold orange.

Days	Wt. of dog kilos	Gold orange grams	URINE Volume 24 hrs. c. c.	Sp. Gr.	Feces
1.	6.18	0.7	200	1025	Diarrhea
2.	6.09	0.7	275	1030	Diarrhea
3.	6.41	...	225	1028	Diarrhea
4.	6.12	...	275	1028	Diarrhea
5.	6.15	...	275	1020	Diarrhea
6.	6.29	0.7	...	....	Diarrhea
7.	6.21	1.4	300	1023	Diarrhea
8.	6.25	2.8	250	1023	Diarrhea
9.	6.25	5.6	...	....	Diarrhea
10.	6.30	11.2	240	1020	Diarrhea
11.	6.24	22.0	...	....	Diarrhea
12.	6.24	33.0	...	....	Diarrhea
13.	6.14	33.0	...	....	Diarrhea
14.	5.86	...	...	....	Diarrhea
15.	...	...	...	....	Diarrhea
16.	6.10	30.0	...	....	Diarrhea
17.	6.10	30.0	...	....	Diarrhea

No samples of urine could be collected in the cage in the usual manner after the eleventh day on account of an incessant diarrhea. The dog manifested no other unusual symptoms than diarrhea. The feces were always colored like the dyestuff itself. The urine also contained the pigment. In spite of the diarrhea throughout the experiment the autopsy revealed no congestion of the intestines. Nothing abnormal could be detected in any part of the body.

Experiment VII a.

Dog: male, weight 5.50 kgs. Diet: meat 115 gms., cracker meal 20 gms., lard 15 gms., bone ash 5 gms., water 275 cc. Dyestuff: ponceau 2 R.

Days	Wt. of dog kilos.	Ponceau 2 R. grams.	Urine Volume 24 hrs. cc.	Sp. Gr.	Feces
1.	5.50	0.5	250	1025	Normal
2.	5.42	0.5	280	1020	Diarrhea
3.	5 20	1 0	300	1017	Normal
4.	5.4)	2.0	300	1015	Normal
5.	5.42	4.0	350	1018	Normal
6.	5.35	8.0	300	1020	Soft
7.	5.30	16.0	290	1035	(dog died)

The urine and feces were always of a deep red color. This experiment proved fatal. There were no special symptoms until the morning of the seventh day when suddenly at 8 a. m. the dog showed signs of paralysis.

The following is a concise record of the symptoms noted from that hour until death :

8 a. m. Rear limbs become paralyzed. Convulsions, frothing at mouth, snapping at various objects especially at the wire of the cage. Lies on his back and moves his legs as if wanting to run. Some symptoms very much like those produced by strychnine.

8.50. Greatly excited and head intensly held back. Frothing has ceased, still tries to bite the cage. Heart is rapid, irregular and weak. Eyes open with anxious look.

9.03. Respiration 17. Heart galloping. Reflexes good. Muscles twitching constantly.

9.15. Gasped, stiffened and relaxed muscles.

9.20. Resting quietly.

9.30. Partially recovered, responded to call, wagged tail, eyes brighter, stood up for a short time but very weak and sank rapidly.

9.40. Tries to stand up, seems dizzy and falls back.

9 46. Stiffening of muscles.

9.49. Heart feeble and irregular; breathing irregular and deep.

9.50. Convulsions again set in. Spine curved and head held far back. Body entirely rigid. Increased reflex sensibility.

9.53. Frothing again conspicuous.

10.05. Bites at wire of cage.

10.10. Again conscious.

10.13. Hyperesthenic.

10.15. Tries to get up but is too weak, bites at cage wire, drags himself around the cage, chews at his own foot and falls together in a heap.

10.28. Spasms similar to those produced by strychnine. Bites at any object.

10.35. Stiffening of body after relaxation of muscles. Seemingly dead.

10.39. Heart still beating feebly.

10.40. Heart stopped beating.

In order to make a further test of this colorant this experiment was repeated.

Experiment VII b

Dog: male, weight 7.84 kgs. Diet: meat 120 gms., cracker meal 32 gms., lard 24 gms., bone ash 8 gms., water 360 cc. Dyestuff: ponceau 2 R.

Days	Wt. of dog kilos.	Ponceau 2 R. grams.	URINE Volume 24 hrs. cc.	Sp. Gr.	Feces
1.	7.82	0.7	320	1013	Normal
2.	7.72	0.7	430	1014	Normal
3.	7.75	1.4	410	1013	Scanty
4.	7.74	2.8	330	1014	Scanty
5.	7.74	5.6	350	1013	Normal
6.	7.75	11.2	350	1014	Normal
7.	7.63	15.0	385	1022	Normal
8.	7.57	22.0	409	1019	Normal
9.	7.50	20.0	415	1020	Soft
10.	7.44	40.0	375	1021	Soft
11.	7.43	60.0	385	1022	Soft
12.	7.45	...	...	....	Normal
13.	7.45	5.0	350	1014	Normal
14.	7.47	5.0	320	1014	Normal
15.	7.47	5.0	370	1016	Normal
16.	7.50	5.0	320	1015	Normal
17.	7.48	5.0	390	1014	Normal
18.	7.49	5.0	350	1014	Normal
19.	7.39	5.0	435	1013	Normal
20.	7.41	5.0	200	1012	Normal

The dog was kept under observation for seven days more without any addition of ponceau 2 R to his food. No irregular symptoms of any kind could be noted. At no time during the experiment did the dog show any signs of illness. On the eleventh day he vomited shortly after eating but ate most of the vomitus. This dog always had a strong appetite and never refused the food tendered him even with the presence of large amounts of colorant.

The autopsy showed nothing abnormal.

Summary of the Results of Experiments I to VII b Inclusive

The results obtained thus far in the above experiments are not sufficient to permit a conclusive answer to the question as to the toxicity of these dyestuffs. In a general way it can be said that judging from the amounts given and the comparatively slight effects produced, these substances can hardly be classed among the virulent poisons. What symptoms they would produce if administered daily in small doses during a very long period or to unhealthy animals is still undetermined.

That all animals are not equally influenced under like treatment was clearly shown in the two experiments on the dosage with ponceau 2 R. One of the animals succumbed after a few days with marked symptoms of poisoning, the other, which received equally large and even larger amounts of ponceau 2 R gave no evidence whatever of any grave disturbances. Neither before nor after death could abnormalities be detected in either animal. It is possible, of course, that the fatal symptoms seen in Exp. VII a were not due to the colorant.

The only outward symptoms caused by the feeding of these dyestuffs, with the exception of the one instance with ponceau 2 R just cited, were such as would be brought about by the administrations of equally large amounts of any of the ordinary saline purgatives. The dyestuffs of the kind used in these experiments are all more or less loaded with potassium or sodium sulphate.

In order to obtain facts for comparison in this connection a control experiment with potassium sulphate[1] was carried out. A dog weighing 8 kg. was given this salt under conditions similar to those of the experiments with the colorants. A dosage of 14 grams gave rise to marked diarrheal stools.

The results of all the dosage experiments are compiled in Table III. It will be seen from the figures that 1.75 gram per kilo of potassium sulphate gave rise to disturbances which in other animals did not appear as a rule until after administration of larger amounts of coloring matter.

TABLE III.

		Wt. of dog.	Duration of experiment. Days.	Grams of substance administered		Grams Dyestuff giving rise to toxic symptoms	
				Total	Per Kg.	Total	Per Kg.
1	Curcumin S	12.3	15	65.53	5.33	10.28	0.83
2	Tartrazin	10.0	10	128.38	12.83	19.60	1.96
3	Naphthol red S	10.8	15	188.33	17.45	25.75	2.38
4	Carmoisin B	12.5	15	177.02	14.15	27.76	2.26
5	Naphthol yellow S	19.0	15	147.56	7.76	30.72	1.60
6	Gold orange	6.2	17	171.1	27.60	33.00	5.32
7a	Ponceau 2 R	5.5	7	32.0	5.82	16.00[2]	2.90[2]
7b	Ponceau 2 R	7.8	20	219.4	28.13	60.00	7.69
8	Potassium sulphate	8.0	5	26.9	3.36	14.00	1.75

Elimination in the Urine

The results of the foregoing experiments indicate that the dyestuffs in question, when administered *per os*, were absorbed and, to a certain extent, passed unchanged into the urine. From the various experimental summaries it will be seen that the specific gravity of the urine was

[1] Of the two, potassium sulphate is only occasionally present. The potassium salt was, however, chosen for this experiment because potassium is the more toxic, and any symptoms induced by this salt would be produced to a lesser degree by the sodium salt.

[2] Proved fatal.

considerably raised during the period of administration and particularly so while excessive amounts of dyes were given. The rise of specific gravity of the urine was undoubtedly due to an increase in its content of inorganic salts, which were derived from the dyestuff. Quantitative determinations of the various urinary constituents were not included in this research. In order to study the extent of elimination of colorant through the kidneys tests were made of the dyeing capacities of the various urines.

For comparison, a series of standards were prepared of each color by dyeing pure wool with 1, 0.5, 0.25, 0.12, 0.06 and 0.03 per cent by weight of dyestuff. This was accomplished by dyeing 1 gram strands of wool in baths consisting each of 10, 5, 2, 1, 0.5 and 0.25 cc. of aqueous 1 per cent. solutions of the respective dyestuffs in 100 c.c. of water and 20 cc. of 10 per cent. sulphuric acid. The dyeing was done at temperatures near the boiling point. To test the applicability of this method to the pigmented urines, 100 cc. of normal urine were used in place of water. It was found that the ingredients of the urine did not interfere with the dyeing. The urine containing a given amount of colorant dyed the wool to practically the same tint as that of the standard.

Fully equipped with a complete set of these standards it is a simple matter by a colorimetric comparison to determine approximately the amount of the dyestuff present in the freshly voided urine. Without giving any figures it can be stated that the amount of coloring matter eliminated by the urine was very slight compared to the amount of coloring matter ingested. By far the largest amount of colorant passed away in the feces. These dyestuffs were not readily absorbed during their passage through the gastro-intestinal tract. When freshly voided, the urine invariably showed the presence of some coloring matter even when small amounts of colorant were taken, and when larger amounts accompanied the food the color of the urine was very marked. At no time, however, was the urine as deeply colored as might be expected after administration of the largest doses of coloring matter.

Some difficulty was encountered in determining the amount of dyestuff in the urine because of the fact that the color of the freshly voided urine frequently changed spontaneously and would not dye wool to the same depth nor to the same shade as before this change had taken place. After standing for a long time the abnormal color of the urine sometimes disappeared entirely. The nature of this change has not as yet been determined. It seems to be due to bacterial action. The same phenomenon may be witnessed when some of the original dyestuff is added to normal urine. Whether the dyestuff under these conditions is subjected to a reduction or an oxidation cannot be stated. Shaking the flask (mixing with oxygen of the air,) seems to accelerate the process. This may, however, be due indirectly to a stimulation of growth of the aerobic bacteria, in turn causing a change of the coal tar color. The presence of a preservative, such as thymol, prevents the change of color.

Although all the dyestuffs under consideration behaved similarly in this respect the effect was most marked with naphthol red S, carmoisin and ponceau 2 R. After administration of the naphthol yellow the urine when voided had a decided yellow color. This yellow color was different from the normal urochrome. The foam of the urine was also yellow and filter paper moistened with the urine showed the presence of some aniline dye. After a time, the color deepened and finally became reddish; on longer standing this color also vanished. Wool was dyed yellow in this urine whether the latter was in the yellow or red condition. After the disappearance of the red color, wool was no longer affected. It was necessary, therefore, to carry out the dyeing tests on the urine with freshly voided samples of the latter or with such as had been treated with thymol immediately after elimination. I have not yet succeeded either by reduction or by oxidation in restoring the artificial color to a sample of urine in which such a change of pigmentation had taken place.

In order to give an idea of the very small amount of coloring matter that was usually eliminated in the urine in these experiments I mention the following data obtained for freshly eliminated urine. The dog given naphthol red S (Exp. V.) received on the seventh day 27.752 grams of this colorant. Twenty-five cc. of uncontaminated and fresh urine dyed one gram of wool to a shade corresponding to that of the 0.5 per cent. standard, that is, the 25 cc. of urine contained five mgs. of dyestuff. During that day the total amount of urine eliminated was 415 cc. No other fraction of this highly colored urine was more deeply tinged than the one selected. Assuming that all fractions contained about the same amount of coloring matter, then the kidneys eliminated during the 24 hours approximately 0.083 gm. of naphthol red S.

Similar calculations for the other dyestuffs gave like results. The figures are interesting in showing the comparatively slight amounts of dyestuff that were eliminated by the kidneys on the days when extremely large doses were administered.

Elimination in the Bile

The elimination of the dyestuffs in the bile was studied on a dog having a complete and permanent biliary fistula[1]. After the wound connected with the fistula had healed the dog was given two grams of dyestuff mixed with a sufficient amount of cracker meal, a small amount of meat and enough water to impart to the mixture a semi-fluid consistency.

[1] I am indebted to Dr. Salant of this laboratory for his kindness in performing the operation.

The bile was then collected[1] during the succeeding 24 hours, but no more dyestuff was added to the daily diet until the third day, when two grams of a different dyestuff were given with the food in the manner just described.

In this way all of the dyestuffs were successively given. Their presence was invariably noticed in the bile a few hours after administration. The bile was so highly and characteristically colored after each administration that the dye tests appeared to be unnecessary for the detection of the colorant. In each case, however, such tests confirmed the conclusion drawn from the general tinctorial effect.

Elimination in the Feces

Whereas the kidneys excreted only small amounts of the dyes, the intestines were, on the contrary, the main channel of their removal. The consistency of the stools was materially affected after the ingestion of the coloring matters, especially when large quantities were given.

In view of the fact that the dyestuffs were secreted in the bile as is indicated above, it can not be stated, from any experimental data thus far acquired, whether any of the dyestuffs were excreted through the intestinal wall. Additional experiments will be necessary to determine this possibility and to demonstrate which sections, if any, of the intestines are active in excreting these substances from the blood through the intestinal wall.

The color of the stools did not always correspond to the original tinge of the dyestuff. The change of color that was noted in various cases (Experiments II, III, IV and V) occurred in the lower part of the gut.

Elimination in the Milk

A bitch in lactation was given with her food daily for three consecutive days two grams of curcumin S. The milk was tested from time to time by applying a piece of white filter paper to a nipple which was pressed gently so as to cause a few drops of milk to exude from it. Not a trace of color could be detected in the milk thus obtained, and none developed on treating the milk spot with a drop of dilute acid. Urine and feces, however, were both colored. The curcumin caused no toxic symptoms in either the mother or the pups.

Subsequently naphthol red S and then carmoisin B were substituted for the curcumin S, and the same procedure as described for the latter was carried out. No color could be detected in the milk at any time after such dosage although the urine and feces were decidedly colored. Carmoisin B, however, caused such pronounced diarrhea that its administration was discontinued after the second dose, to be again resumed after

[1] During the collection of the bile the dog was suspended in a specially designed holder. This apparatus was shown at the exhibition given under the auspices of the New York Academy of Sciences and will be described elsewhere in the near future.

one days intermission. The pronounced diarrheal condition which had somewhat abated during the interim returned after another administration of the dyestuff. Without repetition of this experiment it would hardly be justifiable to ascribe these symptoms to the carmoisin B, especially as a previous experiment on a normal dog gave entirely different results with such a small dose as two grams.

Remarks on the Influence of Coal Tar Colors on Peptic Digestion in Vitro

Gudeman[1] has stated that the coal tar colorants which he examined did not in general inhibit peptic or pancreatic digestion *in vitro* when present in proportions of 1:400 or less of egg albumin. He also ascribes a certain food value to the synthetic dyestuffs. It was therefore of interest to determine whether the colorants considered in this investigation in any way influenced digestion. Experiments were made with fibrin in pepsin-hydrochloric acid. The same pepsin preparation was used throughout.

Solutions containing 5 per cent., 2.5 per cent., 1.25 per cent., 0.62 per cent., 0.31 per cent., and 0.16 per cent. of the various dyestuffs were prepared. One gram of moist fibrin was used for each test. Digestion was carried out at 37° and allowed to continue for several hours *until the fibrin of the control mixture was completely digested.* The concentration of pepsin was the same for each test, 0.05 gm. of "Pepsin aseptic, Parke, Davis and Co." in 100 cc. of the digestive mixture. The results were so striking that the effects of the dyestuffs could be seen at a glance and weighing the undigested fibrin was unnecessary. All of the dyestuffs almost completely inhibited peptolysis in concentrations of 0.62 per cent. or more. Digestion was inhibited in each case at a concentration of 0.31 per cent. of the dye whereas at 0.16 per cent. practically no retardation could be noticed.

Gudemann takes no account of the concentration of the dyestuffs, which is naturally an important determining factor in this connection.[2] The rate of digestion also depends to some extent upon the character of the protein.[3] Proteolysis may be retarded by many substances provided they are in solution. The products of digestion when present in sufficient concentration will alone inhibit digestion. A given amount of dyestuff influences digestion according to its concentration, not especially in proportion to the food it accompanies. Gudeman failed to mention the volume or concentration of his digestive mixtures.

Fujitani[4] has found that many substances which are commonly con-

[1] Gudeman: This Journal 27, 1430 (1905).

[2] The failure of Gudemann to mention the volume of his digestive mixtures or the percentage of dyestuff makes it impossible to compare in any detailed way his data with mine.

[3] Berg and Gies: J. Biol. Chem. 2, 544 (1907).

[4] Fujitani: Arch. Intern. Pharmacodyn. 14, 1-37 (1905).

sidered to be relatively non-toxic, such as sodium chloride, sodium and potassium sulphates, sugar and also tea and coffee infusions even in low concentrations, exert marked inhibitory influences on peptolysis *in vitro*. Numerous salts even in low concentration have a similar effect.[1] It is evident, therefore, that specific deleterious effects on digestion *in vitro* cannot be accepted as indications of *general toxicity in vivo* or *vice versa*.

In Gudemann's particular experiment on the effects of coloring matters on digestion, acetic acid was used in place of hydrochloric acid, the acid accompanying pepsin in normal gastric juices. The reason for choosing the organic acid was, as stated by Gudeman, that "its solvent action on many colors was found to be less than that of the mineral acids." Previous to this statement the same author mentions that he has found sulphuric and phosphoric acids to act as well as hydrochloric acid. No experiments are advanced to substantiate this statement.

The experiments of Gudeman in which acetic acid was used were carried out under most unfavorable conditions. As the colorants were incompletely dissolved, inhibitory influences could evidently only be attributed to the dissolved portions of them. Gudeman's results in this regard are not representative of the total inhibitory powers of these substances on peptic digestion *in vitro*, in the proportions given by him.

Gudeman's choice of acetic acid was unfortunate from another standpoint. Rate as well as extent of proteolysis is least in weak acids[2] such as acetic acid, all other conditions being equal. Recently Berg and Gies[3] have shown that even among the strong acids there are surprising inequalities in the efficiency with which they co-operate with the enzyme in peptolysis. The feebly dissociated acids (organic acids) are not well[4] adapted to activate the pepsin on account of their low degrees of dissociation.

Few acids are inferior to acetic acid in this respect, and Gudemann's results in this connection are of little value on that account.

Conclusions on the toxicity of substances cannot be drawn without reserve, from the effects of such substances on *artificial* digestion. In the process of normal gastric digestion the active principles are being constantly replenished and the digested portions removed, thereby greatly favoring proteolysis. Again, substances which inhibit digestion *in vitro* may stimulate digestion in the stomach by favorably affecting secretory, peristaltic or other conditions.

All of the colorants used in these experiments contained some "filler,"

[1] Schutz: Uber die Hemmung der Pepsinwirkung durch Salze. Beitr. Chem. Physiol., **5**, 407 (1904).

[2] Gies: Am. J. Physiol. 1903, **8**; Pr. Am. Phys. Soc., p. 34.

[3] Berg and Gies: Pr. Soc. Exp. Biol. Med., **4**, 17 (1906).

[4] Berg and Gies: J. Biol. Chem. **2**, 526 (1907).

as already mentioned. So many relatively innocuous substances inhibit digestion *in vitro*, that in these cases the retardation may have been caused by the colorless filler and not by the dyestuff. Further study will be necessary before this point can be settled.

The remarks of Gudemann to the effect that the synthetic colors are *digested* is misleading. What are the products of digestion of these substances? Their digestion can surely not be compared to either that of proteins, fats or carbohydrates. I subjected 0.2 per cent. solutions of these dyestuffs to the influence of pepsin hydrochloric acid during a prolonged period but could not notice any change in their dyeing effects on wool. Gudemann does not mention his methods of determining the *food value* of his colorants.

The same author raises objection to feeding experiments on the ground that substances are thereby introduced greatly in excess of the amounts generally found in foods and that the ill effects "are liable to be due to this excess and in long continued experiments due to a cumulative action of the excess." Surely, if excessive amounts have a cumulative action small amounts may also finally show toxic effects due to retention and accumulation of the poison. To declare a substance entirely innocuous would require evidence as to its nontoxicity both to normal and diseased persons after its long continued administration in both small and large doses. The most extreme contingencies would have to be provided for. The above objections to feeding experiments are therefore not valid. It is hoped that a study of the effects on metabolism of some of these substances will help to further elucidate the subject.

Summary

(1) Several commercial organic dyestuffs (curcumin S, tartrazin, naphthol red S, carmoisin B, naphthol yellow S, gold orange and ponceau 2 R) were studied as to their general effects on dogs when administered in varying amounts and during fairly long periods (two weeks).

(2) None of these dyestuffs under the conditions above indicated exhibited any marked degree of toxicity. There was only one fatal result, which may have been due to influences independent of the action of the colorant.

(3) The dyestuffs were all excreted in part unchanged, with the feces and to a slight extent in the urine. Their presence was demonstrated in the bile but they were not secreted in the milk (bitch).

(4) Peptic digestion experiments have shown that these dyes like many other substances inhibit peptolysis *in vitro*. The interference noted may have been due to the associated inorganic matter.

I desire to express my thanks to Dr. William J. Gies for having proposed this work and acknowledge with pleasure numerous valuable suggestions during its progress.