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A Toxic Factor in Tissues in Cases of Nonspecific Ulcerative Colitis.*

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A substance which is toxic and often lethal for mice has been extracted from the liver and lungs of persons who had nonspecific ulcerative colitis.

Methods. The lungs and livers of persons who died of nonspecific ulcerative colitis, as well as numerous other control diseases, were saponified and the nonsaponifiable fraction[1] was tested for toxicity by subcutaneous injection into mice. The tissues were obtained at necropsy, chilled to ice box temperature immediately thereafter, and preserved by freezing or in a volume of ethanol equal in ml to the weight of the tissue in grams. The animals were of both sexes, of various ages, and of 3 different strains. These 3 factors seemed to have no influence on the results of the tests for toxicity. The extracts were suspended in a fatty solvent. The liver extracts were suspended in sesame oil so that the dose of 500 mg injected into each mouse was contained in a total volume of 1.0 cc. The lung extracts were administered in a dose of 250 mg per mouse, made up in tricaprylin so that the final volume was 1.0 cc. The number of mice used in each experiment was governed by the amount of extract available; it was, of necessity, small.

Results. The toxic factor was recognized by the condition which it induced in the mice, characterized by shock, collapse, and coma, with or without twitching and convulsions, usually terminating fatally. The first sign given by the mice was often scratching of the nose or twitching of some part of the body. Sometimes they lapsed gradually into coma. In other experiments the reaction was ushered in with excitement and intermittent convulsions. The symptoms began between five minutes and one hour after injection, and resulted fatally in from 10 minutes to 4 hours; death usually occurred between 30 and 60 minutes after injection. Mice which had been in deep coma sometimes recovered, regaining normal behavior between the third and fourth hours.

Necropsies revealed no recognizable anatomical abnormality. The lungs were not over-inflated, and on microscopical examination they showed no fat embolism.

Three out of 4 extracts obtained from livers in cases of nonspecific ulcerative colitis were highly toxic, producing shock in every mouse and death in nearly all. (See Table I.) Controls consisted of extracts prepared from 23 livers, representing a variety of common major diseases. Sixteen were entirely nontoxic. Six extracts were possibly very mildly toxic, one mouse succumbing to each. One extract, from a cirrhotic liver, killed 3 mice of 15 injected, but other extracts from cirrhotic livers were nontoxic. In all, 299 mice were injected with the 23 control extracts, with only 9 deaths.

Extracts of lungs from 3 cases of nonspecific ulcerative colitis were tested. Two were not toxic in the doses used while the third was very potent. This extract, when retested 15 months later, was still highly toxic indicating that the toxic factor was stable. Fifty-nine control lung extracts were injected into 451 mice. There were no deaths in shock.

In only one case of nonspecific ulcerative colitis were extracts obtained from both the liver and the lungs. Both proved to be nontoxic, accounting for the one nontoxic liver and one of the 2 nontoxic lungs. This result was not surprising because death in this case was not that of typical nonspecific ulcerative colitis. The patient had had an ileostomy 18 months before death with resultant improvement of the colitis, and she died from peritonitis following a plastic operation on the ileostomy, having at that time a clinical remission and only a little ulceration of the colon.

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1 Steiner, Paul E., *Am. J. Path.*, 1941, **17**, 667.

TABLE I.
Toxicity of Extracts of Human Liver and Lung in Cases of Nonspecific Ulcerative Colitis and Controls.

Exper.	Diagnosis	Wt of tissue, g	Wt of extr., g	Amt inj. per mouse, mg	No. of mice inj.	Acute deaths (4 hrs)	% acute (4 hrs)
Liver 4977	Nonspecific ulcerative colitis	1246	5.1	500	10	9	90
" 5734	" " "	1790	11.4	500	9	9	100
" 5734	(Acid-soluble fraction)	—	—	13	8	2	25
" 5734	(Ether- " "	—	—	500	6	6	100
" 5906	Nonspecific ulcerative colitis	1646	6.6	500	6	4	67
" 5906	(Acid-soluble fraction)	—	—	9	6	5	83
" 5906	(Ether- " "	—	—	500	6	2	33
" 5947	Nonspecific ulcerative colitis	745	3.3	500	7	0	0
23 Control livers	Misc. diseases	—	—	500	299	9	3
Lung 5525	Nonspecific ulcerative colitis	528	1.5	250	5	5	100
" 5749	" " "	681	1.7	250	4	0	0
" 5947	" " "	728	0.7	250	3	0	0
59 Control lungs	Misc. diseases	—	—	250	451	0	0
Infant lungs	Neonatal deaths	2052	14.7	250	18	17	94
" "	" "	—	—	125	26	5	19

Studies on the Nature of the Toxic Factor. Attempts were made to determine the nature of the toxic factor. Hypoglycemic shock was ruled out by a normal blood sugar level (110 mg %) in a mouse which had been in severe shock for about 30 minutes (performed by Dr. Martin Goldner). Fat embolism was excluded by histological studies, by the controls, and by the acid extracts to be described.

Attempts were made to separate the shock-producing principle from the crude extracts by chemical methods in 2 experiments. The original nonsaponifiable residues representing 2 toxic extracts (Liver 5734 and Liver 5906) were dissolved in ether, and extracted 5 times with 1 Normal sulfuric acid. The combined extracts in each experiment were neutralized with barium hydroxide, filtered, and the filtrate evaporated to dryness. These extracts gave negative colorimetric tests for histamine,[2] but gave positive colorimetric tests for tyrosine or tyramine. Mice were then injected with these acid extracts, each animal receiving that quantity obtained from 0.5 g of the original toxic residue. The ether-soluble fractions were tested in parallel to determine if any toxicity persisted in them. The tests showed that the toxic principle was now divided; in Liver 5734 about half of it was found in the acid-soluble fraction, and in Liver 5906 over half of it was in this fraction.

[2] Koessler, K. K., and Hanke, M. T., *J. Biol. Chem.*, 1919, **39**, 497.

Since the acid-soluble fraction contained no histamine according to the method used for testing, but did possibly contain tyramine, this substance was tested in pure form for toxicity in mice. Mice were injected with 0.135 mg of tyramine hydrochloride, which was the amount found in each mouse-dose in the acid-soluble fraction of Liver 5734. This amount was without noticeable effects on the animals, as was a dose 4 times as large (0.54 mg). It seemed from these experiments that while the toxic acid-soluble fractions contained tyramine that this substance was not present in sufficient amounts to account for all of the toxicity. It must be attributed to other toxic amines or to other substances.

Discussion. The toxic factor described in extracts of human liver and lung in cases of nonspecific ulcerative colitis was not found exclusively in this disease. It or a toxin which resembles it closely, was occasionally found in dilute or in mild form in extracts of liver in other diseases. It was not found in extracts from numerous control adult lungs, but 2 different extracts prepared from large amounts of pooled lungs (46 lungs, 2,052 g) from stillborn infants were toxic. In contrast to this observation was the behavior of an extract of pooled livers from stillborn infants, which did not contain the toxic factor under comparable conditions.

The factor was not related to the presence of necrotic tissue in the body. Many of the

persons had cancer, and indeed some of the lung extracts which were not toxic were obtained from lungs containing primary carcinomas or tumor metastases.

The possibility that the toxic factor was absorbed from or through the denuded surfaces (ulcers) in the bowel was entertained. This idea was supported by the absence of toxicity in the livers from one case of quiescent nonspecific ulcerative colitis, several cases of ulcerated carcinoma of the colon, and one case of superficial ulcerative colitis believed to have been the result of a gold-sodium-thiosulphate reaction, because in none of these colons were the ulcerations extensive. By this explanation, however, the toxicity found in the infant lungs remains unexplained, if it be assumed that the toxic substances are identical. The same objection applies to regarding this toxic factor as the cause of the ulcers during its excretion through the mucosa.

Summary. A toxic factor was demonstrated in extracts of the liver and lungs in cases of nonspecific ulcerative colitis. A factor with similar toxic properties was present in lesser amounts or in milder form in an occasional control extract of liver, and in lungs of stillborn infants, but not in adult control lungs or infant livers.

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The Flea *Malaraeus telchinum* a Vector of *P. pestis.*

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In the course of an extensive survey instituted in an important section of the San Francisco Bay region in order to determine the source of the *P. pestis* demonstrated in pools of fleas, 1,356 rodents (Meadow mice—*Microtus californicus* 952, Deer mouse—*Peromyscus maniculatus* 347, Brown rat—*Rattus norvegicus* 40, and Harvest mice—*Reithrodontomys megalotis* 17) were trapped. Their ectoparasites were collected and adequate samples of fleas, both males and females, were cleared and identified. The species determinations and host data are listed in Table I.

It was found that *Malaraeus telchinum* was by far the most numerous of the fleas found on *Microtus californicus,* and that this flea outnumbered *Nosopsyllus fasciatus* about 2 to 1 on *Rattus norvegicus. Opisodasys nesiotus* was slightly more numerous than *Malaraeus telchinum* on *Peromyscus maniculatus.*

Since *Malaraeus telchinum* was the only species of flea found to parasitize all 3 species of rodents known to be spontaneous hosts for *P. pestis,* it was deemed imperative to undertake transmission experiments. Such experiments were prompted by the observations of Eskey and Haas[1] who noted that 74 *Malaraeus telchinum* fed on a plague-infected guinea pig were apparently unable to transmit *P. pestis* to guinea pigs despite the fact the flea developed blockage.

Wheeler and Douglas[2] using white mice as sources for the infection reported the failure of *Malaraeus telchinum* to transmit *P. pestis* in mass feeding experiments on white mice. In the experiments herewith reported, *Peromyscus maniculatus, Microtus californicus,* and the white mouse were used as a source for the infection. The pools of the fleas so infected were permitted to feed on *Microtus californicus.*

Ten experiments have thus far been completed, and 4 positive transmissions have been obtained as follows:

(1) Seventy-five fleas were fed on a moribund plague-infected *Peromyscus maniculatus.* Six of the fleas were triturated in a mortar

1 Eskey and Haas, *Pub. Health Bull.,* No. 254, 1940, 42.

2 Wheeler and Douglas, PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 65.