'aggregated' mice which have been described here were not evident unless the (^{3}H) d-amphetamine was injected into the mice; i.e., these could not be shown when brains were merely homogenized in the presence of (^{3}H) d-amphetamine.

Discussion. In this report we have presented data which indicate that the action of d-amphetamine of decreasing selectively the entry of glucose carbon atoms into the brains of 'isolated' mice[2] is correlated with an increased retention of (^{3}H) of injected (^{3}H) d-amphetamine by particles in the P_2 fractions of their brains. Perhaps the most likely explanation is that due to a more rapid rate of metabolism in 'aggregated' (hyperactive) mice (Figure 1), d-amphetamine is metabolized and excreted more rapidly and therefore does not exert such a pronounced effect on brain metabolism (see also reference [8]). It should be noted that the results presented here, as well as those in our previous reports[2,8], were obtained with mice subjected to 'prolonged' periods of differential housing and therefore are not expected to be in accord with results of 'short-term' studies[9,10]. The data obtained in this study, and in other studies involving environmental effects on metabolism and drug action, may be subject to variation due to a number of factors such as: 1. changes in body or ambient temperature[11]; 2. diurnal rhythmicity[12,13]; 3. size of test compartment[14]; 4. strain of mice[4]; 5. conditions of housing prior to experiments; 6. seasonal influences. In any case, it seems reaconable to suspect that social isolation of animals, including man, may cause changes in the central actions of addictive drugs[15].

Résumé. La rétention de (^{3}H), après l'injection de (^{3}H) d-amphétamine chez des souris, fut plus marquée dans des particules synaptiques quand les animaux avaient été soumis à un isolement prolongé.

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Pathogenesis of Gastro-Intestinal Symptomatology During Poisoning by *Amanita phalloides*

During the poisoning of humans by *Amanita phalloides* the first symptoms appear after 10–24 h – violent abdominal pains, vomiting and persistent diarrhoea.

So far it has been excluded[1] that these gastrointestinal symptoms might be due to amanitins or phalloidins, which are the cytotoxins responsible for damage to liver and kidney in *Amanita phalloides* poisoning[1–3]. This belief derives from the finding that administration of these toxins in common experimental animals (mouse, rat and guinea-pig), whether orally or by injection, does not produce gastro-intestinal symptoms. This reasoning, however, takes no account of the fact that even a total extract of *Amanita phalloides* produces no such symptoms in these animals, whereas the earlier literature[4] informs us that total extracts administered orally or by injection to dogs do produce gastro-intestinal symptoms. Therefore it is the dog, not the mouse, rat or guinea-pig, which should be chosen for investigations on the gastro-intestinal symptoms produced in Man by *Amanita phalloides*.

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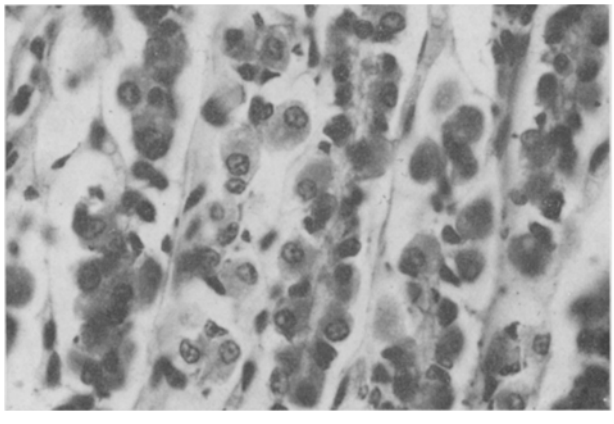

Fig. 1. Stomach of a dog killed by α-amanitin. Mucosa of the fundus. Atrophy of chief cells with picknosis of nuclei. Em. Eos. × 525.

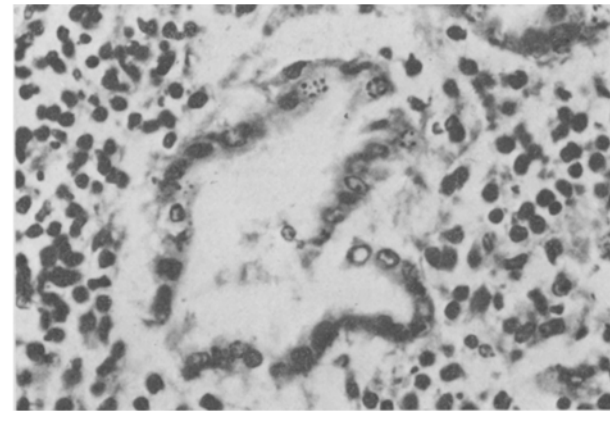

Fig. 2. Brunner's gland in the duodenum of a dog killed by α-amanitin. Marked changes of nuclei. Em. Eos. × 525.

The aim of the present study has been to find out wheter α-amanitin, the main cytopathic toxin in *Amanita phalloides*[3], produces gastro-intestinal lesions in dogs leading to vomiting and diarrhoea. 4 dogs (henceforth, 1, 2, 3 and 4) and 4 Swiss albino male mice were used. Before administration of α-amanitin the dogs were observed for 2 weeks, to ensure that they were in good health.

Injection with α-amanitin was performed on dogs 1, 2 and 3 (8.5 kg male, 8 kg female, 6 kg male, respectively), the doses being 0.6 mg/kg s.c., 0.3 mg/kg s.c. and 0.5 mg/kg i.p. respectively. Dog 4, a 12 kg male, was given no α-amanitin; it was sacrificed to provide a sample of morphologically normal cells from the alimentary canal of a dog.

Death in the first 3 dogs was caused by α-amanitin, in the 4th by vein-cutting under ether anaesthesia. The 4 mice were killed 24 h after i.p. injection of 0.5 mg/kg of α-amanitin.

Autopsy was performed on all 4 dogs and 4 mice. For histological examination, pieces of liver, kidney, and stomach, and of various parts of the intestine were removed and were fixed in 4% formalin buffered to pH 7 and in Ruffini's liquid No. 3. After being embedded in paraffin, sections were stained with hematoxylin and eosin.

Dogs 1, 2 and 3 began to suffer from vomiting and diarrhoea 9–11 h, and died 20–24 h, after injection of α-amanitin. At autopsy the mucosa of stomach and intestine in all 3 dogs was found to be reddened. The intestinal mucosa was covered by a catarrh-like substance which, in the jejunum and, especially, ileum, appeared to be hemorragic.

The histological examination of the organs of dogs 1, 2 and 3 revealed severe lesions in some cells in the stomach, duodenum, jejunum and ileum. The surface epithelial cells of the stomach, and the chief and parietal cells of the glands of the fundus and body of the stomach were all damaged (Figure 1). In the small intestine, the surface cells and the cells of the glands, including Brunner's glands in the duodenum (Figure 2 and 3), were affected. The main damage, a vesicular appearance, pycnosis and karyorrhexis, was found in nuclei.

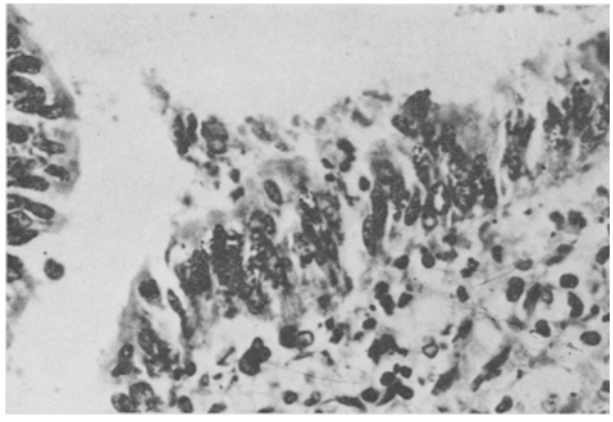

Fig. 3. Surface epithelial cells of jejunum of a dog killed by α-amanitin. Marked changes of some nuclei. Em. Eos. ×525.

The ratio between numbers of damaged and apparently undamaged cells was much higher in the duodenum than in the stomach, jejunum and ileum; in the latter organs most cells had a normal appearance, and lesions were found only in single cells or small groups of cells scattered through the mucosa. No inflammation of the gastro-intestinal mucosa was observed in any of the 4 mice killed after administration of α-amanitin; nor did histological examination reveal signs of damage in the epithelial cells of their alimentary canal.

Our research shows that the injection of α-amanitin into dogs leads to vomiting and diarrhoea. In contradiction to previous opinion on this subject, it is therefore highly probable that the gastro-intestinal symptoms observed in Man during poisoning by *Amanita phalloides* are due to this toxin (and to the other amanitins, which act in the same way[5]).

The cytopathic effect of α-amanitin is due to its capacity to inhibit RNA-polymerase II in eukariotic cells[1,3,6]. There are, however, some cells which are not damaged by this toxin. There is evidence that this resistance is due to α-amanitin being unable to penetrate into these cells[7].

The difference between the effects of α-amanitin on dogs and mice is probably due to its being able to penetrate into some of the cells in the gastro-intestinal canal in the former but not the latter. In the dog the probable order of events is: 1. α-amanitin enters into some of cells in the gastric and intestinal mucosa, damaging them, 2. this damage leads to gastro-enteritis, with vomiting and diarrhoea.

The incapacity of α-amanitin to penetrate into the cells of the gastro-intestinal canal in mice would explain both the absence of gastro-intestinal lesions and also why the mouse, which is highly sensitive to injected α-amanitin, is highly resistant to it when administered orally[1].

The fact that in the dog the proportion of damaged cells is much higher in the duodenum than in other parts of the gastro-intestinal canal could be due to a possible elimnitation of injected amanitin through the bile.

Riassunto. L'iniezione di α-amanitina causa nel cane una gastroenterite con vomito e diarrea. Pertanto, contrariamente a quello che si credeva, la sintomatologia gastrointestinale nell'avvelenamento umano da *Amanita phalloides* è con molta probabilità provocata da questa tossina.

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[8] The authors thank Prof. Th. Wieland for the generous gift of α-amanitin. This investigation was supported by a grant from the Consiglio Nazionale delle Ricerche.

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