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THE ACTION OF CYSTEAMINE AND SYNKAVIT ON GASTRIC EMPTYING IN THE NORMAL AND IRRADIATED RAT

BY

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A radiographic method for studying the delay in gastric emptying in the rat caused by whole body irradiation has been applied to the investigation of two compounds which modify the effects of irradiation. The method may be of general use in the study of the action of drugs. Synkavit, which can potentiate the lethal effects of irradiation on tumours, caused a delay in gastric emptying. When Synkavit and irradiation were combined, a simple summation of the delays produced by each of these agents occurred. Cysteamine, which gives some protection against the lethal action of x-rays, also caused a delay in gastric emptying. It did not protect against the action of x-rays on stomach emptying.

X-irradiation gives rise to a delay in gastric emptying in the rat (Goodman, Lewis, and Schuck, 1952; Billings, Bennett, and Burlingame, 1954; Swift, Taketa, and Bond, 1955). The effect of doses as low as 20 r. whole body irradiation (some 3% of the LD50/30 days) has been demonstrated by using serial radiographs to follow the progress of a barium meal in the unanaesthetized rat (Hulse, 1957). Barium meals were used by Smith and Irving (1955) to assess the effect of sodium salicylate on stomach emptying in rats, and it appeared that the routine used in the irradiation experiments was suitable for testing the effect of substances which might alter gastric emptying. Cysteamine [β-mercaptoethylamine] and Synkavit [sodium salt of 2-methylnaphth-1:4-ylene di-(dihydrogen phosphate)] have been tested in this way, cysteamine because it is known to reduce the lethal effects of radiation and Synkavit because it sensitizes tumours to the action of x-rays.

METHOD

The details of the technique for giving the barium meal and taking the radiographs have been described elsewhere (Hulse, 1957). The essential points are that the rats were not forcibly fed with the opaque meal and not unduly restrained during radiography. Ease in feeding was achieved by training the rats to take a thick paste of malted milk from a spatula so that when due for experiment they willingly accepted a paste of malted milk mixed with barium sulphate (Horlicks Shadow Meal). A tunnel-like plastic container with opaque sides and one perforated end was used to keep the rat in a suitable position without frightening it. The rat readily entered the container which was closed by sliding a piece of perspex into one of two slots, according to the size of the animal.

Litters of four male rats of an inbred albino strain aged between three and six months were used throughout the experiments. The various treatments given were randomly allocated within the litters.

After a litter had been fed with the barium sulphate mixture, the compound to be tested was given as an intraperitoneal injection to one pair of rats whilst the other pair received the same volume of saline, also given intraperitoneally. The injections were made speedily and gently in order to disturb the rat as little as possible. One animal of each pair was given an appropriate dose of x-rays either before or after the compound under investigation was given. The other two rats acted as unirradiated controls.

Radiography

Control radiographs to assess the degree of filling of the stomach at the start of the experiment were taken as soon as possible after the barium meal and before the irradiations. If, as in the case of Synkavit, a period of time had to elapse between administering the test substance and irradiation a further pre-irradiation film was taken. After irradiation, radiographs were taken at half-hourly intervals from the time of irradiation for 3 hr. and then at hourly intervals until 8 hr. had elapsed. During each radiographic exposure, the rats received a dose of approximately 0.05 r.; the total irradiation through taking radiographs was therefore less than 1.5 r.

Treatments

Synkavit.—Six litters of rats were used. The dose, given immediately after the barium meal, was 1.0 ml. (equivalent to 10 mg. of the free ester). The irradiation (20 r. of *x*-rays) was given half an hour after the administration of the Synkavit.

Cysteamine.—Three different combinations of cysteamine (Becaptan, Labaz) and irradiation were used. With each combination, two parallel series of experiments were performed; in one the cysteamine was given within 5 min. before, and in the other within 5 min. after irradiation. The three dose schemes were 20 mg./kg. of cysteamine and 30 r. of *x*-rays (12 litters), 100 mg./kg. of cysteamine and 20 r. of *x*-rays (eight litters), and 100 mg./kg. of cysteamine and 100 r. of *x*-rays (eight litters).

Radiation Procedures

These were all whole-body irradiations and the irradiation factors were as described previously (Hulse, 1957). All the non-irradiated animals were subjected to sham irradiations.

RESULTS

The results have been assessed in the same manner as in the previous investigation. The degree of fullness of the stomach was judged by comparison with the appearances of the radiograph taken immediately after feeding, and five stages (100, 75, 50, 25, and 10%) were easily distinguished. An average of the degree of fullness of all the rats in an experimental group was obtained and graphs were constructed relating the average degree of fullness to the time after irradiation. In order to compare different treatments, the average of the combined scores from 5 to 8 hr. inclusive after irradiation was calculated for each type of treatment. By subtracting the average of the appropriate controls from that of the experimental groups an expression was obtained for the net effect of each procedure (Tables I and II).

TABLE I

THE EFFECT OF SYNKAVIT ON STOMACH EMPTYING IN THE IRRADIATED RAT

Average fullness of stomach expressed as % at 5 to 8 hr. after irradiation (mean ± S.E.).

Treatment	Observed Effect	Net Effect of Treatment
Saline injection and no irradiation	5±1	—
Saline injection followed by 20 r. of *x*-rays ..	34±11	29±11
Synkavit injection and no irradiation ..	42±9	37±9
Sum of net effects of 20 r. saline and Synkavit without irradiation..	—	66±14
Synkavit injection followed by 20 r. of *x*-rays	69±9	64±9

Effect of Synkavit.—The injection of 1 ml. of Synkavit by itself gave rise to a delay in gastric emptying slightly greater than 20 r. of *x*-rays, and when Synkavit and 20 r. of *x*-rays were both given a delay in stomach emptying was produced which

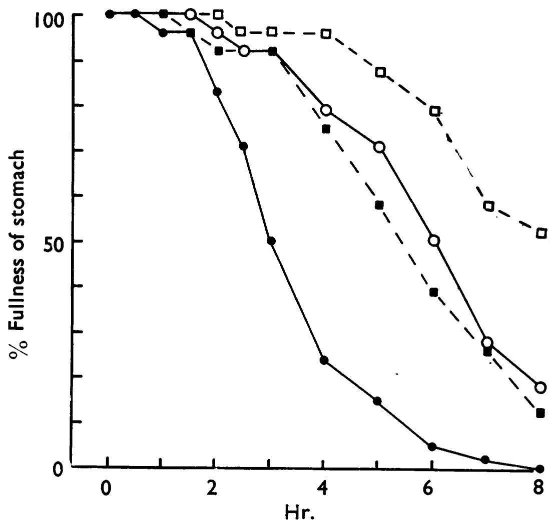


FIG. 1.—The effects of Synkavit and 20 r. of whole body *x*-irradiation on the pattern of gastric emptying. Each curve represents an average of six animals. ——●—— no irradiation, saline injection; – –■– – whole body *x*-rays, saline injection; ——○—— no irradiation, Synkavit injection; – –□– – whole body *x*-rays, Synkavit injection.

TABLE II

THE EFFECT OF CYSTEAMINE AND *X*-IRRADIATION ON STOMACH EMPTYING IN THE RAT

Average fullness of the stomach expressed as % at 5 to 8 hr. after irradiation (mean ± S.E.).

	Observed Effects			Net Effects of Treatments		
Dose of irradiation (r.)	30	20	100	30	20	100
Dose of cysteamine (mg./kg.)	20	100	100	20	100	100
Treatment:						
None (saline injection only)	8±2	12± 2	15±7	—	—	—
X-irradiation (saline injection only)	32±6	33± 5	82±6	24±6	21± 5	67±10
Cysteamine (no saline)	52±5	93± 4	90±4	44±6	81± 4	76± 9
Combined net effects of cysteamine and irradiation..	—	—	—	68±9	(102± 7)	(143±13)
Cysteamine followed by *x*-irradiation	81±8	84±12	95±5	73±9	72±1	80± 9
X-irradiation followed by cysteamine	86±4	81±12	95±5	78±5	69±12	80± 9

was greater than either alone (Fig. 1). The net effect of the combined treatment was not significantly different from the sum of the net effects of the treatments given singly ($P > .9$; Table I).

Effect of Cysteamine. — Cysteamine alone caused a delay in gastric emptying, the degree of delay being dependent on the dose. The effect of 100 mg./kg. was very marked (Figs. 3 and 4), whilst that of 20 mg./kg. was less marked but nevertheless quite definite (Fig. 2). During preliminary experiments with various doses of cysteamine it was found that 15 mg./kg. gave a delay only slightly less than 20 mg./kg. and 25 mg./kg. a delay much nearer that of 100 mg./kg.

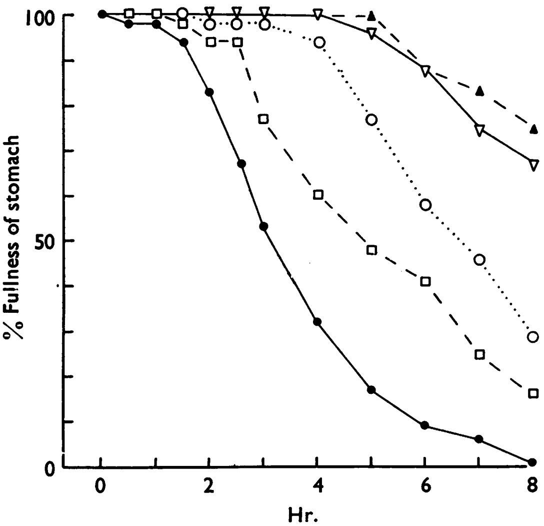


FIG. 2.—The effects of cysteamine (20 mg./kg.) and 30 r. of *x*-rays single and combined (with the cysteamine given either before or after irradiation) on the pattern of gastric emptying. The curves for the animals given no irradiation and saline, 30 r. and saline, and no irradiation and cysteamine are the averages of twelve animals. The curves for cysteamine and irradiation combined are each averages of six animals. ——●—— No irradiation, saline injection; — — □ — — whole body irradiation, saline injection; · · · · O · · · · no irradiation, cysteamine injection; ——▽—— whole body irradiation after cysteamine injection; — — ▲ — — whole body irradiation before cysteamine injection.

When 20 mg./kg. of cysteamine was combined with 30 r. of *x*-rays a much greater delay in stomach emptying was produced than either treatment by itself regardless of the order in which the combined treatments were given. The 5 to 8 hr. averages of the combined treatments are almost the same as the sum of the individual treatments (P = .7 to .8 and .3 to .4 ; Table II).

The higher dose of cysteamine (100 mg./kg.) when combined with 20 r. of *x*-rays, regardless of order, gave a delay in gastric emptying much in excess of that produced by 20 r. (Fig. 3) and not significantly different from that of 100 mg./kg. alone (Table II). The combined effects of

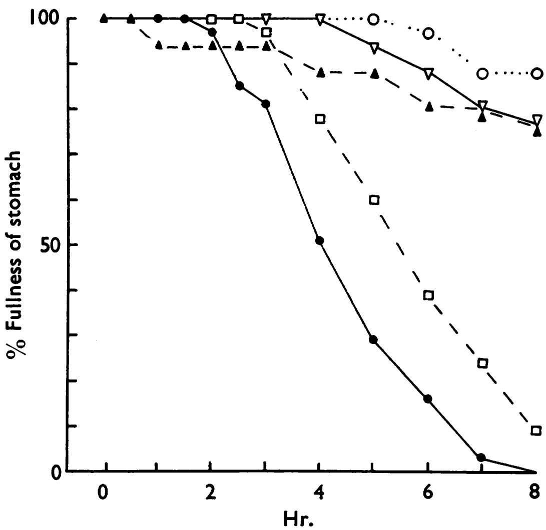


FIG. 3.—The effect on stomach emptying of a high dose of cysteamine (100 mg./kg.) and 20 r. of *x*-rays singly and combined (cysteamine either before or after irradiation). The curves of the cysteamine and irradiation combined both represent averages of four animals. The other curves are each averages of eight animals. ——●—— No irradiation, saline injection; — — □ — — whole body irradiation, saline injection ; · · · · O · · · · no irradiation, cysteamine injection ; ——▽—— whole body irradiation after cysteamine injection; — — ▲ — — whole body irradiation before cysteamine injection.

100 mg./kg. of cysteamine and 100 r. of *x*-rays are also independent of the order in which they are given and statistically are similar to the effects of the separate treatments (Fig. 4). The nearly maximal response of the individual treatments using the highest doses prevents a valid comparison between the sum of their net effects and the net effect of the combined treatments (Table II).

Effects of Saline Injections.—An intraperitoneal injection of saline had no appreciable effect on stomach emptying. The previous investigation (Hulse, 1957) included studies of control and irradiated rats which had not received any injections. It is possible to compare these directly with the saline injected animals in the cysteamine experiments. In each case, the shapes of the curves (Figs. 2, 3, and 4) are similar to those obtained previously in the uninjected rat.

During the examination of the radiographs, it was noted that within a few hours of the large dose of cysteamine being given the shape of the stomach changed and became more globular (Fig. 5). The appearances suggest that there may be a loss of tone in the walls of the stomach after this drug. A tendency towards this change in shape was frequently seen with the smaller dose of cysteamine, but neither Synkavit nor x-rays altered the normal contour of the stomach.

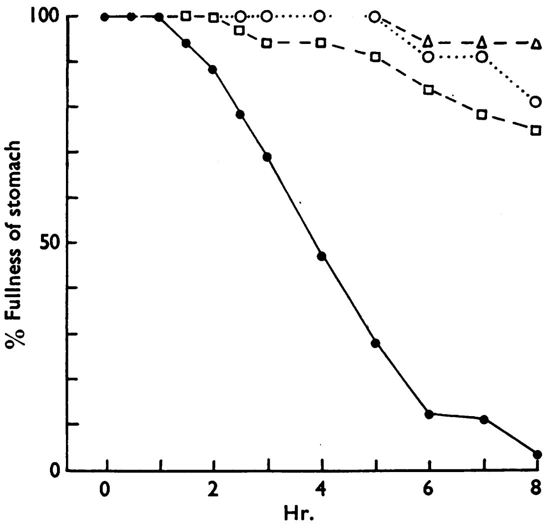


FIG. 4.—The effect on stomach emptying of a high dose of cysteamine (100 mg./kg.) and 100 r. of x-rays singly and combined. The curves are each averages of eight animals; exactly similar curves were obtained for the combined treatments whether the cysteamine was given before or after irradiation. ——●—— No irradiation, saline injection; — — □ — — whole body irradiation, saline injection; · · · · O · · · · no irradiation, cysteamine injection; — — Δ — — whole body irradiation, cysteamine injection.

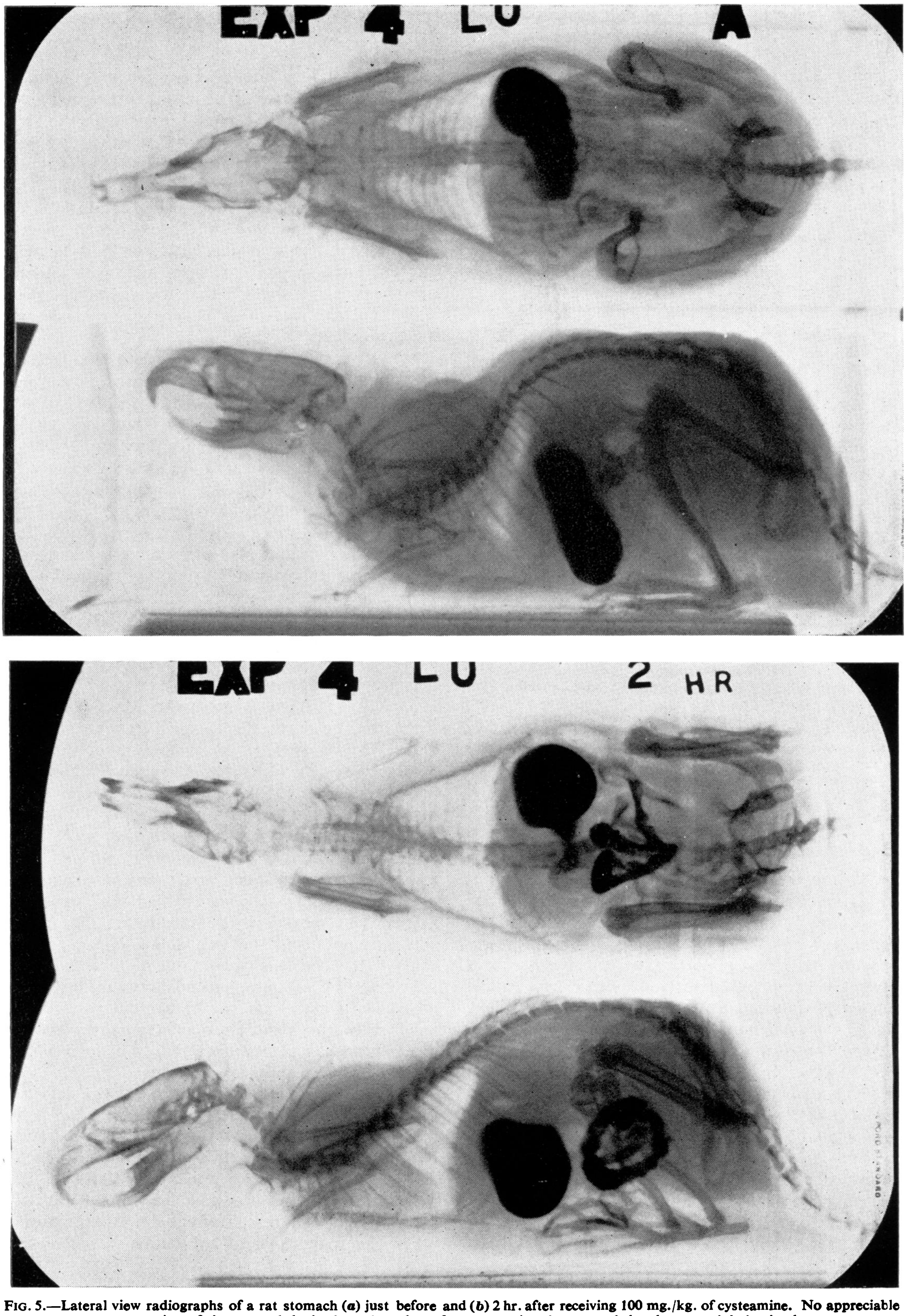
DISCUSSION

The delay in gastric emptying produced in the rat by ionizing radiations has been amply confirmed since the original observations of Ely and Ross (1947) on neutron irradiation, but the mechanism by which it occurs is still not known. Various other agents are known to give rise to a delay in stomach emptying including muscle ischaemia, traumatic shock, severe haemorrhage, changes in environmental temperature, and injections of sodium salicylate (Whiteley and Green, 1952 ; Swift *et al.*, 1955 ; Geiger and Pinsky, 1956 ; Smith and Irving, 1955). The suggestion that post-irradiation gastric delay is therefore part of a non-specific response to stress (Swift *et al.*, 1955) has been disproved by demonstrating that adrenalectomy does not alter the response (Hulse, 1957). The post-irradiation vomiting seen in other species has recently been studied by neurological methods. Chinn and Wang (1954) found that bilateral destruction of the emetic chemoreceptor zone in the region of the area postrema prevented post-irradiation vomiting in the dog, and Brizzee (1956) confirmed the findings in the monkey and also found that supradiaphragmatic vagotomy inhibited post-irradiation vomiting. Borison (1957) has extended this type of work, using cats as the experimental animal, and concluded that the phenomenon is initiated in the abdomen and that the afferent impulses traverse both the vagus and the dorsal spinal roots, and that the segmental pathway involves innervation which is independent of the sympathetic distribution.

Synkavit has been used experimentally and therapeutically as a means of enhancing the effects of irradiation on tumours (Mitchell, 1954, 1955). The results reported here show that Synkavit has a similar action on stomach emptying to that of irradiation. When irradiation and Synkavit were combined the result was a simple summation of their individual effects (Table I).

Cysteamine, when given before irradiation, reduces the numbers of deaths occurring in groups of experimental animals during the first few weeks after exposure, but when given after irradiation has no effect (Bacq and Alexander, 1955). The drug has, however, been given after therapeutic irradiation in man to combat the symptoms of radiation sickness (Bacq and Herve, 1952 ; Bacq, 1953, 1954 ; Bacq, Dechamps, Fischer, Herve, Le Bihan, Lecomte, Pirotte, and Rayet, 1953), but Court Brown (1955) was unable to confirm the beneficial results which had been reported. In the present experiments cysteamine has been found to give a delay in gastric emptying in the rat similar to that produced by irradiation itself. With none of the schemes of dosage and with neither of the orders of treatment was there any reduction of the irradiation effect when the irradiated animal was treated with cysteamine, in fact the evidence points to the two delays being additive (Table II). As rats are unable to vomit, gastric delay may be analogous to post-irradiation vomiting seen in man. If so the present results offer support to the contention of Court Brown that cysteamine has no therapeutic action in radiation sickness.

A widely accepted explanation for the action of cysteamine in reducing the lethal effects of x-rays is that it competes for the free radicals produced by radiation and is itself oxidized in the process. As both irradiation and cysteamine cause a delay in gastric emptying it would be expected that any such antagonistic action would be at a maximum when the magnitude of the delay caused by the



a

b

FIG. 5.—Lateral view radiographs of a rat stomach (*a*) just before and (*b*) 2 hr. after receiving 100 mg./kg. of cysteamine. No appreciable emptying of the stomach had taken place, but after cysteamine the stomach has become globular in shape.

cysteamine and the irradiation was about equal. The doses of 30 r. of *x*-rays and 20 mg./kg. of cysteamine were chosen with this in view, but the two agents are in this case clearly additive in their effects. Although these results are not sufficient to dogmatize upon, they do at least suggest either that delay in gastric emptying is not initiated by the action of free radicals or that the theory of free radical competition is inapplicable in the case of gastric emptying as presumably it is in the case of certain genetic effects of irradiation (Kaplan and Lyon, 1953a and b).

It has been suggested that cysteamine does not reduce the apparent radiation damage by interfering with the initial interaction between radiation and living tissues but affects, by some biochemical mechanism, the biological repair process (Bond and Cronkite, 1957). No evidence, however, for such an action was found in the case of radiation injury of the intestinal epithelium (Mole and Temple, 1957). If, as is quite probable, recovery from radiation-induced gastric delay is represented by the stomach emptying which eventually takes place, it is clear that from this aspect also cysteamine offers no protection.

Given proper precautions, of which gentle handling and training of the rats are probably the most important, serial radiographs after a barium meal can be a simple method of investigating agents which may effect stomach emptying. The method may be particularly useful for testing compounds designed to alleviate post-irradiation nausea and vomiting. Other methods of making numerical comparisons can be used instead of the 5 to 8 hr. average. For instance the time taken for the stomach to reach half-full (50% emptying time) is a possible measure if the time of observation is extended to well over 8 hr. Care, however, would have to be taken that the dose of irradiation from taking the larger number of radiographs was not excessive. Whenever this method of studying stomach emptying time is used, the sensitivity of the system to irradiation makes it important that a check should be made on the amount of irradiation received during radiography and precludes the use of screening.

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