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# Effect of X irradiation on the active transport of glucose through the intestine of the rat *in vivo* *

by
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Former observations (1, 3, 8, 9, 17, 18) had already revealed alterations in the intestinal absorption of glucose subsequent to the irradiation, even when the results did not eliminate influences on gastric emptying, the speed of the passage through the intestine and other factors. DETRICK et al. (7) saw that rats which had received 600 r showed a decrease in the absorption of glucose at 3 or 6 days, measuring the transport through segments of intestine *in vitro* MOSS (19) also verified a remarkable decrease 72 hours after irradiating the intestine with 700 r. More recently, and also measuring the absorption of glucose *in vitro*, ZEHNDER (31) observed that by irradiation with 800 r the absorption of glucose decreases after 7 hours, increases at 24 and decreases once again at 48, a result confirmed by HEHL and WILBRANDT (11), who attribute the first inhibition to an effect on the adrenals. The inhibition during the first hours was not observed, on the other hand, by SULLIVAN (26), who verified, nevertheless, the decrease after three days of exposure of the intestine to 900 or 1500 r. An initial increase at 22 hours, followed by a later increase, has been reported by WESEMANN et al. (30).

In a previous work (16) we demonstrated that the irradiation of anesthetized rats with lethal doses of X rays (1200-3000 r) provoked serious alterations in the transport capacity of the intestine *in situ*, throughout the six hours following the irradiation, as also the radioprotective effect of cysteamine (10 mg/100 mg) when it was present during the irradiation in the segment of jejunum used for measuring the absorbent capacity.

The diversity of the results to be found in the bibliography dealing with the effect of the ionizing radiations (X rays) on the capacity of absorption of glucose by the intestine, besides the paucity of data concerning the effects of radioprotective substances on these alterations, has led us to study this subject more thoroughly. To this end we have measured the absorption during

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the 96 hours following the X irradiation with a dose of 600 r, by means of an *in vivo* technique (25) which permits us to discard gastric emptying and under conditions in which the glucose has to pass into the blood against a gradient of concentration.

## Material and Methods

The experiments have been carried out with adult male white rats, whose weights were between 200 and 300 g, of the Wistar strain, subjected to a standard diet.

The animals, not anesthetized and having fasted for 24 hours previously, were irradiated inside perforated polythene tubes, using a Siemens* apparatus of 200 kV and 10 mA and with a Cu filter of 0.5 mm.

According as the experiments suggested, irradiation was practised on the whole animal, the abdomen only or the whole body except the abdomen. The areas which were not intended to be irradiated were protected under layers of lead with a thickness of 5 mm.

In all these cases the animals were subjected to a dose of irradiation of 600 r and their capacity for active transport was determined at different times. For this purpose different groups of animals were irradiated, which were then operated on at the most suitable time for beginning the first absorption 2, 8, 24, 48 and 96 hours after the irradiation.

The active transport of d-glucose has been studied by the Sols and Ponz successive absorption technique (25), under anesthesia with urethane (120 mg/100 g). In all the experiments was made use of 2.77 mM d-glucose solution, in ClNa at 9 ‰ (154 meq $Na^+$/l), with an initial volume to be absorbed of 10 ml and a repletion pressure of the intestine of about 8 cm of water, with cannulated segments of the jejunum of about 20 cm in length (28).

On each animal four successive absorptions were carried out, lasting 20 minutes each, with no longer interval between one and another than the time needed for washing the intestine and changing the solutions. The rectal temperature of the animals was kept constant (± 0.5 °C) throughout the experiment.

The determinations of the residual glucose were made according to the method of Somogyi (24).

The results are expressed in the tables in average values of each group of animals with their standard errors (21) in micromoles of glucose absorbed per centimetre of intestine (28). In the figures the absorption is given as so much per cent of the normal.

In the experiments with radioprotective substances we have utilized 2-mercaptoethylamine hydrochloride (cysteamine, Calbiochem), in a 5 % solution in sterilized bi-distilled water which was administered by intraperitoneal injection, ten minutes before irradiating, in a dose of 10 mg/100 g.

## Results *

### A. *Effect of the irradiation of the whole body*

A study was made, in the first place, of the constancy and level of the capacity of active transport of glucose in a large group of non-irradiated animals, subjected to the same manipulations as those which were exposed to the action of the X rays. The intestinal absorption

* We wish of express our thanks to the Department of Physical Therapeutics of the Faculty of Medicine of Barcelona University for the facilities afforded for the irradiation of the animals.

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of glucose remains practically constant throughout the four successive absorptions.

Two hours after the irradiation with 600 r the absorption is diminished, an effect which is rather more noticeable eight hours after having irradiated the animals. At 24 hours, on the other hand, it has increased. 48 hours after the irradiation the absorption is normal, but it then falls noticeably, at 96 hours (Table I).

In each of these groups the effect becomes apparent in the four successive absorptions carried out on each animal, in the which absorptions the quantity of sugar absorbed remains practically constant. We can thus observe an initial decrease, followed by an increase in the absorbent capacity (24 hours after irradiation), and later an evident inhibition, which becomes particularly apparent at 96 hours (Fig. 1).

B. *Effect of the irradiation of the abdominal region only*

In order to ascertain whether the effects provoked by the irradiation of the whole body were due to an action on the whole organism or whether they were also produced by irradiation of the abdominal region alone, we proceeded to irradiate the rats with 600 r, keeping the whole body protected with lead except the abdomen.

The intestinal absorption was studied 8, 24 and 96 hours after irradiating the

TABLE I

Effect of total body irradiation with 600 r on the active transport of glucose through the intestine of rat.
Glucose 2.77 mM with NaCl at 9 ‰ (154 meq. $Na^{+}$/l); absorption time: 20 minutes. Irradiation on animal without anaesthesia.

| No. Animals | Post-Irrad. hours | Successive absorptions (μM/cm) | | | |
|---|---|---|---|---|---|
| | | 1st. Abs. | 2nd. Abs. | 3rd. Abs. | 4th. Abs. |
| 18 | — | 0.32 ± 0.01 | 0.31 ± 0.01 | 0.32 ± 0.01 | 0.30 ± 0.03 |
| 6 | 2 | 0.26 ± 0.02 | 0.28 ± 0.03 | 0.26 ± 0.03 | 0.26 ± 0.01 |
| 6 | 8 | 0,22 ± 0.01 | 0.22 ± 0.01 | 0.23 ± 0.02 | 0.24 ± 0.01 |
| 6 | 24 | 0.43 ± 0.02 | 0.45 ± 0.02 | 0.43 ± 0.01 | 0.44 ± 0.02 |
| 6 | 48 | 0.32 ± 0.02 | 0.30 ± 0.02 | 0.31 ± 0.01 | 0.31 ± 0.02 |
| 6 | 96 | 0.19 ± 0.02 | 0.18 ± 0.02 | 0.19 ± 0.02 | 0.18 ± 0.02 |

TABLE II

Effect of abdomen irradiation with 600 r on the active transport of glucose through the intestine of rat.
Glucose 2.77 mM with NaCl at 9 ‰ (154 meq. $Na^{+}$/l); absorption time: 20 minutes. Irradiation on animal without anaesthesia.

| No. Animals | Post-Irrad. hours | Successive absorptions (μM/cm) | | | |
|---|---|---|---|---|---|
| | | 1st. Abs. | 2nd. Abs. | 3rd. Abs. | 4th. Abs. |
| 6 | — | 0.33 ± 0.01 | 0.32 ± 0.02 | 0.31 ± 0.01 | 0.32 ± 0.02 |
| 6 | 8 | 0.24 ± 0.01 | 0.24 ± 0.02 | 0.24 ± 0.03 | 0.23 ± 0.04 |
| 6 | 24 | 0.38 ± 0.02 | 0.38 ± 0.02 | 0.37 ± 0.02 | 0.39 ± 0.02 |
| 6 | 96 | 0.19 ± 0.07 | 0.18 ± 0.01 | 0.17 ± 0.03 | 0.18 ± 0.02 |

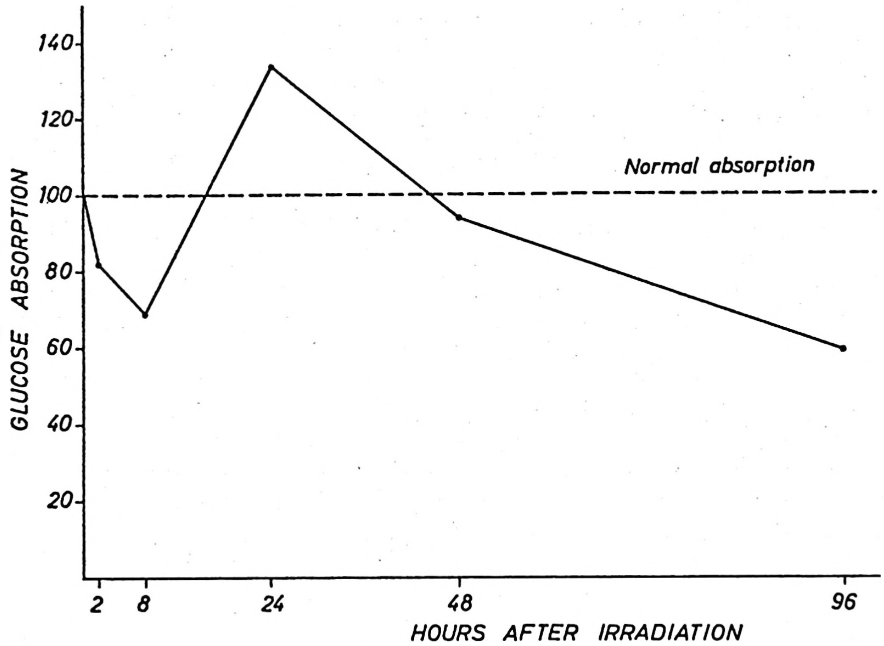


FIG. 1. Changes of the intestinal absorption of glucose 2.77 mM, provoked by X irradiation (total body) with 600 r, according to the time that follows the irradiation (2 to 96 hours). Control absorption, 100.

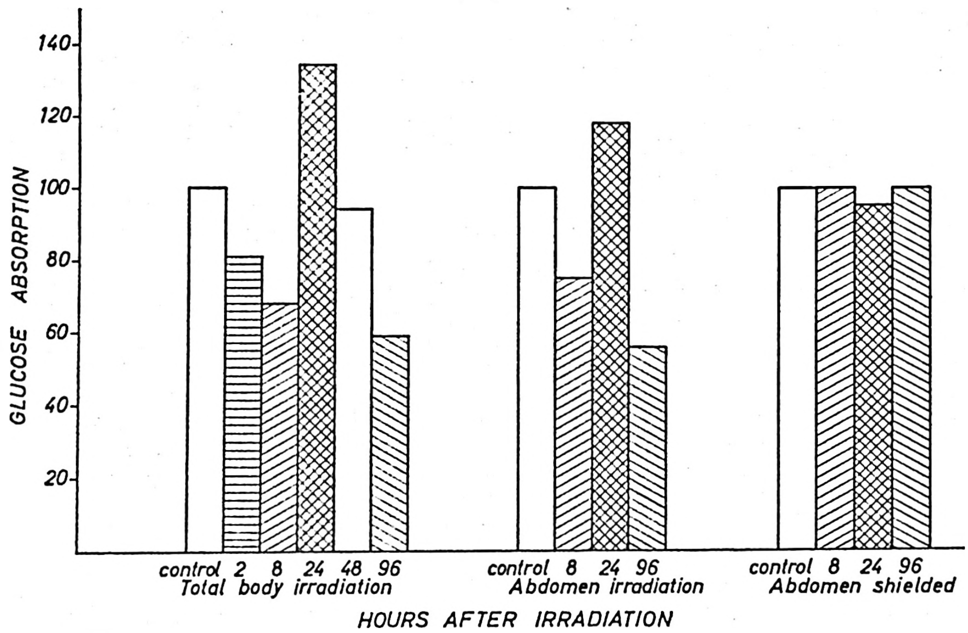


FIG. 2. Effect of X irradiation (600 r) on the intestinal absorption of glucose (2.77 mM) at 8, 24 and 96 hours after irradiating, according to the parts submitted to irradiation. Control absorption, 100.

animals, periods of time to which corresponded more apparent alterations (see Table I).

As may be observed in Table II and figure 2, the irradiation of the abdomen alone gave rise to effects practically identical to those found after irradiation of the whole body.

### C. *Effect of the irradiation of the whole body except the abdomen*

The preceding results were complemented by making a study of the effect of the irradiation (600 r) of the whole body except the abdominal region, which was shielded with lead. As in the previous case, the absorption figures were taken 8, 24 and 96 hours after the irradiation.

In Table III and figure 2 it can be clearly seen that in these conditions irradiation with 600 r does not produce significant variations in the active transport of glucose through the intestine of the rat.

### D. *Radioprotective effect of cysteamine on the alterations of the active transport of glucose provoked by X irradiation*

We thought it of interest to study whether cysteamine, a radioprotective substance, was capable of protecting the animal from the effects which the ioni-

TABLE III

Effect of total body irradiation, excepting the abdomen, with 600 r on the active transport of glucose through the intestine of rat.
Glucose 2.77 mM with NaCl at 9 °/₀₀ (154 meq. $Na^+$/l); absorption time: 20 minutes.
Irradiation on animal without anaesthesia.

| No. Animals | Post-irrad. hours | Successive absorptions ($\mu$M/cm) | | | |
|---|---|---|---|---|---|
| | | 1st. Abs. | 2nd. Abs. | 3rd. Abs. | 4th. Abs. |
| 6 | — | 0.31 ± 0.03 | 0.32 ± 0.04 | 0.31 ± 0.02 | 0.33 ± 0.04 |
| 6 | 8 | 0.32 ± 0.01 | 0.32 ± 0.02 | 0.32 ± 0.03 | 0.32 ± 0.03 |
| 6 | 24 | 0.30 ± 0.01 | 0.30 ± 0.02 | 0.31 ± 0.02 | 0.30 ± 0.04 |
| 4 | 96 | 0.32 ± 0.02 | 0.33 ± 0.01 | 0.32 ± 0.01 | 0.32 ± 0.01 |

TABLE IV

Radioprotective effect of cysteamine. Intestinal absorption of glucose in total body irradiated rats (600 r).
Glucose 2.77 mM with NaCl at 9 °/₀₀ (154 meq. $Na^+$/l); absorption time: 20 minutes.
Intraperitoneal cysteamine (10 mg/100 g), 10 minutes before irradiating. Irradiation on the animal without anaestesia.

| No. Animals | Post-irrad. hours | Successive absorptions ($\mu$M/cm) | | | |
|---|---|---|---|---|---|
| | | 1st. Abs. | 2nd. Abs. | 3rd. Abs. | 4th. Abs. |
| 4 | — | 0.32 ± 0.02 | 0.30 ± 0.03 | 0.31 ± 0.03 | 0.31 ± 0.04 |
| 4 | 2 | 0.34 ± 0.02 | 0.34 ± 0.02 | 0.34 ± 0.02 | 0.34 ± 0.02 |
| 4 | 8 | 0.28 ± 0.02 | 0.30 ± 0.02 | 0.29 ± 0.02 | 0.30 ± 0.02 |
| 4 | 24 | 0.30 ± 0.01 | 0.31 ± 0.01 | 0.33 ± 0.04 | 0.30 ±0.01 |
| 4 | 48 | 0.32 ± 0.01 | 0.32 ± 0.01 | 0.30 ± 0.04 | 0.31 ± 0.01 |
| 4 | 96 | 0.29 ± 0.02 | 0.31 ± 0.03 | 0.29 ± 0.02 | 0.31 ± 0.03 |

zing radiations produce in the intestinal absorption of glucose.

In non-irradiated animals, the intraperitoneal injection of cysteamine (10 mg/100 g) did not change the glucose absorption capacity.

Various groups of rats were injected with the same dose of cysteamine ten minutes before being exposed to the irradiation (the whole body, 600 r), and a study was made of their capacity for the active transport of glucose 2, 8, 24, 48 and 96 hours after the irradiation. In all the cases it could be observed (Ta-

TABLE V

Radioprotective effect of cysteamine. Intestinal absorption of glucose in abdomen irradiated rats (600 r).

Glucose 2.77 mM with NaCl at 9 °/₀₀ (154 meq. $Na^+$/l); absorption time: 20 minutes. Intraperitoneal cysteamine (10 mg/100 g), 10 minutes before irradiating. Irradiation on the animal without anaestesia.

| No. Animals | Post-irrad. hours | Successive absorptions (μM/cm) | | | |
|---|---|---|---|---|---|
| | | 1st. Abs. | 2nd. Abs. | 3rd. Abs. | 4th. Abs. |
| 4 | 8 | 0.30 ± 0.32 | 0.30 ± 0.02 | 0.31 ± 0.01 | 0.32 ± 0.03 |
| 4 | 24 | 0.32 ± 0.02 | 0.31 ± 0.01 | 0.29 ± 0.01 | 0.31 ± 0.02 |
| 4 | 96 | 0.32 ± 0.01 | 0.32 ± 0.01 | 0.31 ± 0.01 | 0.31 ± 0.03 |

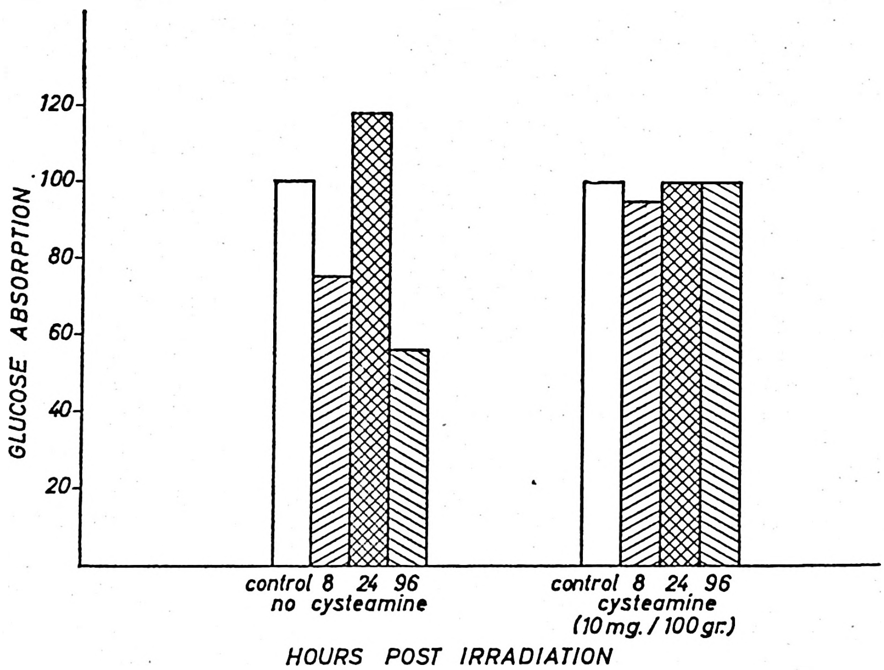


FIG. 3. Radioprotective effect of cysteamine as regards the action of the radiations (600 r, only abdomen) on the intestinal absorption of glucose 2.77 mM at 8, 24 and 96 hours from irradiation. Control absorption, 100.

ble IV) that the intestinal absorption of glucose was not significantly distinguishable from that of the normal, non-irradiated animals.

Cysteamine, in the dose indicated, completely protects the rats from the effects of the irradiations on the intestinal absorption of glucose, at least during the 96 hours to which our experiments extended.

A study was also made of the radio-protective effect of cysteamine in the experiments of irradiation of the abdominal region only. The absorption was measured at 8, 24 and 96 hours.

It appears evident (Table V, Fig. 3) that the protective action of cysteamine in experiments with irradiation of only the abdominal region is also complete, since the absorption of glucose in these conditions remains absolutely normal.

## Discussion

The dose of ionizing radiations utilized in all our experiments is about $DL_{50}$ (27) and therefore its action on the digestive tube is quite clear (4, 23, 29).

Our results indicate that the irradiation with 600 r alters in very evident fashion the active transport of glucose through the intestine of the rat *in vivo*. We have observed, first, an inhibition (2 to 8 hours), which later turns into an increase (24 hours) and then falls progressively (24 to 96 hours).

These disturbances must be caused by the effect on abdominal organs, as they appear after irradiation of the whole body or of the abdomen only and are not manifest when the whole body except the abdomen is irradiated.

ZEHNDER (31) and HEHL and WILBRANDT (11) had also found an inhibition followed by a recovery and, later, a fall in the first 48 hours. The latter did not find the exaltation which ZEHNDER (31), WESEMANN et al. (30) and we ourselves all found at 24 hours. The inhibition which can be remarked in the first hours following the irradiation has been attributed to the adrenals (11), since it does not appear in animals in which these glands have been extirpated. This first phase of inhibition was not observed in the experiments of SULLIVAN (26).

The second phase of inhibition, which presents itself after the first 24 hours, is more intense and lasts longer than the first, with absorption minima which vary according to the authors (7, 20, 26), but which can be calculated to appear 3 or 4 days after a $DL_{50}$, or even later.

It is very probable that the first, transitory, inhibitory reaction, which is to be observed within the first hours, is the result of a physiological response, of a neuroendocrine character, in which the adrenal organs are surely involved, as is thought by HEHL and WILBRANDT (11), and that it does not depend on a morphological lesion of the epithelium, which would need a longer time to become apparent. Nevertheless, it cannot be doubted that the functional capacity of the epithelium for the active transport of sugars is diminished, since the inhibition is manifest both in experiments *in vivo* and in those *in vitro*.

The second phase of inhibition, on the other hand, must be already intimately linked to the alterations in the intestinal epithelium which are provoked by the irradiation of the intestine itself. This inhibition appears, as we have seen in our experiments, thanks to irradiation either of the whole animal or of the abdomen only, and with or without adrenals (11) and irradiating only intestine (19).

The observations made with the aid of the electron microscope (6, 10) reveal that during the first 24 hours following a dose of 525 r or even 3,000 r no morphological changes of any importance are to be noticed in the epithelium of the villi, even when the cells of the crypts are affected and are also observed focal edema. The third day, on the other hand, the villi appear to be dehydrated and lower, the epithelium presents abnormal or immature cells with sparse, thick, short microvilli, as well as various alterations in mitochondria and other structures. Allowing for some differences in time and dose, similar descriptions have been made by other authors (12, 13). The alterations in the epithelium of the villi derive from those already provoked very shortly after the irradiation in the cells of the crypts.

A small part of the reduction in the capacity of intestinal absorption of glucose has been explained as being a consequence of the decrease in the amount of food taken by the rats during the first and second day following the irradiation (20).

On the other hand, the correlation between morphological changes of the epithelium and absorbent function, reckoning from 24-48 hours, is far from being complete (7), and it is not unusual to observe a marked functional alteration, even with partially restored epithelia.

Given the close links existing between the active transport of sodium and that of sugars, it is possible that the primary effect of the radiations is exercised on the active transport of sodium, which in turn would have a secondary influence on that of sugars. Indeed, acute intestinal radiation death has been attributed to the loss of water and electrolytes (2, 22). The irradiation provokes a grave loss of the total sodium of the organism (15), partly because it is not re-absorbed by the intestine (14). CURRAN et al. (5) have revealed that the active transport of sodium is very sensitive to irradiation.

The experiments of radioprotection with cysteamine, in any case, have shown excellent efficacy when it is administered intraperitoneally ten minutes before the irradiation. The radioprotection was practically complete, whether the whole body was irradiated or only the abdomen, and this confirms our previous observations (16) in experiments in which the cysteamine was administered in solution placed in the intestinal lumen. Although reports had already been made on the radioprotective action of cysteine and of AET with respect to the effect of radiations on intestinal absorption (26), the efficacy of cysteamine had not previously been tested.

## Summary

A study has been made, using the Sols and Ponz successive absorption technique, of the effect of X irradiation of animals (600 r) on the capacity of active transport of glucose (2.77 mM) by the intestine of the rat *in situ*, through time (2 to 96 hours), as also of the radioprotective effect of cysteamine.

The irradiation of the whole body quite clearly alters the absorption. An initial decrease can be noticed (2 to 8 hours), followed by an increase (24 hours) and later a second phase of inhibition up to 96 hours.

These disturbances must be attributed to an effect on organs of the abdominal cavity, since they are produced both by irradiation of the whole body and by that of the abodminal region alone, whereas, on the other hand, they cease to appear when the animals are irradiated with the abdomen protected.

Cysteamine (10 mg/100 g, intraperitoneal injection ten minutes before the exposure) exercises a perfect radioprotective action.

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