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Effect of Stress upon the Healing of Wounds in Rats.* (20495)

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Although the phenomena related to stress have been intensively studied in the past few years, the effect of stress upon the healing process has never been clearly determined. Stress results in increased secretion of adrenal cortical hormones(1). The administration of ACTH or cortisone, in sufficient dosage, inhibits the rate of healing(2,3). Whether or not the increased endogenous secretory activity of the pituitary-adrenal axis in response to stress can depress the rate of healing of an experimental wound, is not established.

Studies concerning the effect of stress upon healing have led to contradictory conclusions. It has been reported(4,5) that if 2 wounds are produced in an animal at different times, the second wound heals more rapidly than the first. On the other hand, it has also been reported(6) that a skin defect, made one or 2 days following a procedure such as gonadectomy, healed more slowly than similar wounds made some time after the operative intervention. Intercurrent infections, located some distance from healing wounds, were observed to slow the rate of healing in a few animals (7). Intramuscular injections of an irritant, sodium ricinoleate, reportedly reduced the rate of healing in dogs on the 7th and 10th postoperative days(8).

In an attempt to clarify the relationship between pituitary-adrenal function and the healing process, a series of experiments was begun in this laboratory in 1951 utilizing the bursting pressure of standard laparotomy

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wounds in rats as an index of the rate of healing.

Method. The technic employed here is a modification of our previously reported method(9) which is objective and withstands statistical analysis. Young Wistar rats, weighing 180-200 g, were submitted to laparotomy with aseptic technic under ether anesthesia. A 3 cm right rectus incision was made and repaired with 6 interrupted 00000 silk sutures through the entire thickness of the abdominal wall. The skin was then sutured with continuous No. 38 stainless steel wire.

On the fifth postoperative day, the animals were again anesthetized with ether. The skin was carefully dissected away from the wound, and the silk sutures removed. Then, through a small stab incision in the left lower quadrant a rubber balloon was inserted into the peritoneal cavity. The balloon was then slowly inflated with air. The pressure of air necessary to burst the abdominal incision was recorded. The end-point has generally been quite sharp and simple to determine. At the conclusion of the wound test, the animals were autopsied. The adrenal glands were carefully dissected out and weighed promptly on a Roller-Smith torsion balance.

Eleven series of observations were made. (Table I) I: Skin incision 6 cm in length over the back, sutured with continuous fine wire 4 days prior to laparotomy. II: Excision of a 6 by 3 cm patch of skin from the back, done 4 days prior to laparotomy. III: Fractures of the femur, knee joint, tibia and fibula bilaterally, produced 4 days pre-laparotomy. IV: Turpentine injection, 1.0 ml, subcutaneously, given in one dose 4 days prior to laparotomy. V: Turpentine, 0.5 ml injections daily beginning 9 days pre-laparotomy and continuing until day of sacrifice. VI: Burn, second degree, 25% of body surface (back), at 73°C for 30 seconds, 4 days pre-laparotomy. VII: Refrigeration at 4-6°C for 4 days prior to laparotomy and continuing until day of sacrifice. The animals were removed from refrigeration only for the duration of the laparotomy procedure. VIII: Epinephrine injections, 0.2 mg daily beginning the day of laparotomy and continuing until day of sacrifice. IX: dl-Thyroxine injections, 0.5 mg daily, beginning 6 days pre-laparotomy. X: Cortisone acetate (Cortone, Merck) injections, 1.0 mg twice daily, beginning 4 days pre-laparotomy. XI: ACTH (Acthar Gel, Armour) injections, 4 units twice daily, beginning 4 days pre-laparotomy. The skin incisions, excisions, burns and fractures were all produced under ether anesthesia.

All animals were maintained in individual cages on an *ad libitum* intake of Purina Laboratory Chow and water. Despite the various injuries, the average body weight of the various groups of rats either increased or remained unchanged during the 10-day experimental period.

Results. The results are summarized in Table I. With the exception of the skin-incision and the single-dose-turpentine stresses, in every case there is a statistically significant depression of the bursting pressure of the fifth day healing wounds. There is a concomitant increase of the adrenal weight in each type of stress in which this factor was studied, as compared with the weight of the adrenal glands in non-stressed laparotomized control rats.

In an attempt to determine for what period of time after initiation of stress this inhibition of healing is manifest, several types of stress were produced at variable intervals of time prior to laparotomy. These results are summarized in Table II. It is apparent that skin incision did not significantly depress the bursting pressure of 5-day wounds at any time interval studied. On the other hand, skin-excision-stress, initiated the day of laparotomy or 4 or 9 days prior to laparotomy, did result in impaired healing. Inhibition of healing did not occur when this type of stress was initiated 15 to 30 days prior to laparotomy. The skin excision wounds were usually healed by the 30th day. When multiple fractures were employed as the stress, depression of the fifth day bursting pressure persisted even 45 days after the fractures were produced.

Discussion. Our results are not in agreement with those of Sandblom(4) and of Young, Fisher, and Young(5). These authors used skin wounds in their experiments. With the use of skin wounds, it is well known that numerous local factors may affect the result,

TABLE I. Magnitude of Stress-Induced Inhibition of Healing as Determined in 5-Day Laparotomy Wounds Following Different Types of Stress.

Nature of stress	No. of rats	Adrenal wt, mg/100 g B.W.	Mean bursting pressure ± S.E.	Statistical analysis* n	t	P
Non-stressed control	27	8.6 ± .2	75.4 ± 2.2	—	—	—
Skin incision, 4 d. pre-lap.	10	9.4 ± .3	66.2 ± 3.7	35	2.08	N.S.
Skin excision, 4 d. pre-lap.	34	10.0 ± .3	60.0 ± 1.5	59	5.25	<.01
Fractures, 4 d. pre-lap.	11	—	60.7 ± 3.4	36	3.53	<.01
Turpentine, 1 cc, 4 d. pre-lap.	10	—	79.0 ± 3.1	35	—	N.S.
Turpentine, .5 cc o.d., 9 d. pre-lap.	11	—	60.9 ± 2.8	36	3.67	<.01
Burn, 4 d. pre-lap.	11	10.0 ± .3	61.3 ± 2.3	36	3.70	<.01
Refrigeration, 4 d. pre-lap.	11	12.5 ± .5	55.3 ± 4.1	36	4.70	<.01
Epinephrine .2 mg, o.d. post-lap.	12	10.8 ± .4	61.0 ± 3.2	37	4.21	<.01
Thyroxine, .5 mg, 6 d. pre-lap.	9	11.6 ± .6	60.7 ± 2.9	34	3.47	<.01
Cortisone, 1.0 mg b.i.d., 4 d. pre-lap.	10	5.8 ± .3	60.0 ± 2.6	35	5.26	<.01
ACTH, 4 u b.i.d., 4 d. pre-lap.	6	15.3 ± .4	53.5 ± 4.0	31	4.18	<.01

* Statistical analysis by the "t-test" of Student as described by Fisher(11). Mean bursting pressure considered significantly different from control when P <.04.
S.E. = Stand. error of the mean. N.S. = Not significant.

TABLE II. Effect on Healing of Stress Initiated at Varying Intervals of the Time Prior to Laparotomy.

	Mean bursting pressure	Diff. from control	Statistical analysis n	t	P
Control	75.4 ± 2.2	0	—	—	—
Skin incision					
At lap.	72.7 ± 5.3	2.7	34	—	N.S.
4 d. pre-lap.	66.2 ± 3.7	9.2	35	2.08	.05
9 d. " "	70.9 ± 2.9	4.5	38	1.18	N.S.
Skin excision					
At lap.	63.5 ± 3.7	11.9	37	2.84	<.01
4 d. pre-lap.	60.0 ± 1.5	15.4	59	5.25	<.01
9 d. " "	56.9 ± 2.0	18.5	59	5.25	<.01
15 d. " "	70.6 ± 2.5	4.8	43	1.4	N.S.
30 d. " "	68.9 ± 3.5	6.5	33	1.4	N.S.
Fractures					
At lap.	57.6 ± 3.6	17.8	34	4.04	<.01
4 d. pre-lap.	60.7 ± 3.4	14.7	36	3.53	<.01
9 d. " "	60.0 ± 2.6	15.4	40	4.32	<.01
15 d. " "	59.6 ± 3.3	15.8	35	3.76	<.01
30 d. " "	52.0 ± 2.3	23.4	34	5.6	<.01
45 d. " "	65.9 ± 1.8	9.5	39	2.76	<.01

a fact which is emphasized by Sandblom. This may be an explanation for the discrepancy. In no instance in our study did any group of animals demonstrate a mean bursting pressure, significantly greater than that of the normal control rats. This is in agreement with current views regarding the healing process as summarized by Harvey(10).

The duration of the period of stress-induced inhibition of healing appeared to depend upon the nature and the magnitude of the stress. With multiple fractures, healing was de-

pressed 45 days after the fractures were produced. Actually, bony union of the fractured femoral shafts had not occurred in many cases by this time. Skin-excision-stress did not result in depression of the bursting pressure of laparotomy wounds if the skin excisions were made 15 days or more before laparotomy.

Two possibilities suggest themselves to explain the stress-induced inhibition of healing. First, there may be competition between the repair process in the area of trauma (fractures or skin wounds) and the healing laparotomy wound for certain, as yet undetermined, metabolites which are essential to the healing process. Second, the induced stress may result in sufficient stimulation of pituitary-adrenal activity so that the increased endogenous secretion of adrenal cortical hormone results in inhibition of the rate of healing. The presence of hypertrophied adrenals following severe stress would favor the second hypothesis.

Summary. 1. A variety of situations, calculated to provide a chronic "stress" stimulus, have been studied in order to determine the effect of stress upon the bursting pressure of standard laparotomy wounds in rats. 2. With the exception of mild stress (skin incisions, single turpentine injection), in each case a depression of the bursting pressure of fifth day laparotomy wounds was observed. This was accompanied by increases in the weight of the adrenal glands at autopsy. 3. The duration of this period of stress-induced depression of healing appeared to vary with the magnitude and the nature of the stress stimulus.

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Hafnium Complexes for Biological Investigation.* (20496)

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A hafnium sodium mandelate complex has been used in a recent study of the distribution and excretion of hafnium in the rat(1). Hf^{181} is one of the relatively small number of readily available isotopes which have a half-life at all suitable for internal irradiation by deposition in the tissues(2). It therefore seemed important to learn whether it is practical to obtain substantially different distribution patterns using other complexing agents. Other workers have shown that the distribution patterns of colloids can be altered by variation in particle size(3,4) and that the excretion pattern of lanthanum can be changed by the use of chelating agents(5). Another reason for interest in other hafnium complexes is the fact that the mandelate preparations are often visibly cloudy at pH 7-8, while the other complexes described subsequently are more

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