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تأثير خلاصة الغدة الصنوبرية في الجمال وبعض مشتقاتها الفعالة (السيروتونين والميلاتونين) على النمو الجسمي والحاجز العصبي الدموى

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أجرى هذا البحث لدراسة تأثير خلاصة الغدة الصنوبرية في الجمال وبعض مشتقاتها (السيروتونين والميلاتونين) على النمو الجسمي . واستخدم في هذا البحث أربعون فأرا حديثي الولادة قسمت الى أربعة مجموعات كل منها عشرة فئران . المجموعة الأولى ضابطة والثانية تم حقنها بخلاصة الغدة الصنوبرية للجمال والثالثة بمادة السيروتونين والرابعة بمادة الميلاتونين . وذلك من اليوم الثاني بعد الولادة ولمدة أربعة أسابيع.

وأظهرت النتائج أن خلاصة الغدة الصنوبرية أو بعض مشتقاتها أحدثت نقصا ذا دلالة معنوية في وزن حيوانات التجارب . وكمحاولة لدراسة السبب لقلة الوزن . هل لأن المواد المحقونة تقلل من شهية الحيوانات فتؤدى الى نقص التغذية أو أنها تؤدى الى قلة افراز هرمون النمو داخل الجسم ؟

أختبرت سلامة الغطاء المحيط بالاعصاب كدليل لعدم تعرض هذه الحيوانات لنقص التغذية ولما وجدت سليمة فان احتمال نقص افراز هرمون النمو نتيجة الحقن هو الاحتمال الأكبر.

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EFFECT OF PINEAL EXTRACT OF CAMEL AND SOME PINEAL ACTIVE SUBSTANCES (SEROTONIN AND MELATONIN) ON GROWTH AND BLOOD-NERVE BARRIER

(With 1 Table & 4 Figs.)

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SUMMARY

To study the effect of pineal extract of camel and or pineal active substances melatonin and serotonin on somatic growth, forty newly born rats were divided into four groups, ten animals each (three experimental and one control). The experimental groups were daily injected with either pineal extract, serotonin or melatonin, starting from day 2 postnatally up to the 28th day. The weights of the injected groups were all reduced below the control values and the decrease was highly significant. To test, if the reduction was due to a matabolic factor or undernutrition, the perineurial barrier was examined by a HRP tracer technique. All experimental animals showed a normal intact barrier function like the control. This means that those animals were not subjected to undernutrition and the cause of weight reduction may be a metabolic factor related to growth hormone.

INTRODUCTION

There is much collecting evidence about a relationship between the pineal gland and somatic growth, each through a different approach. The results were semmingly compatible with the idea that it restricts growth.

REITER et al. 1968 & REITER 1970, applied an indirect method. They removed the eyes from rats during their rapid growth phase. This led to lowering of body weight. The effect is exagerated if the animals are also rendered anosmic. In both the singly and doubly sensory deprived rats, the animals grew almost normally if they also had been pinealectomised (REITER and ELLISON, 1970 and REITER et al. 1971).

Pinealectomy, as a direct method to study the effect of pineal gland on growth was done by (MALM et al. 1959), it caused acceleration of bodily growth in rats.

Also; administration of some pineal constituents diminished growth hormone synthesis or secretion and as a result restricted growth (SORRENTIONO et al. 1972).

SMYTHE and LAZARUS (1973) showed that the pituitary gland of cat has the ability to concentrate peripherally administered melatonin. They suggested that melatonin has a direct action on pituitary gland to block the release of growth hormone.

On the other hand, whereas the pineal active substance melatonin decreased the maturation of the reproductive organs in male rats it failed to inhibit the growth of animals (SORRENTION et al. 1971).

In fact, data implying the relationship between the pineal gland and its active principles on somatic growth seems rather new and have not yet been established. Indeed, there is still some conflicting results concerning this subject (RONNELKEIV and MC CANN 1978).

The present work was designed to study the effect of either pineal extract or its active substances, serotonin and melatonin on somatic growth by a direct approach, viz; through the regular injection into newly born rats during the period of their rapid growth. The cause of delay in growth has been always thought to be a metabolic factor related to growth hormone.

The other part of this investigation was a histochemical tracer technique as a speculation on the mechanism of growth delay, to answer the question of whether the delay of growth in injected animals was due simply to anorexia leading to undernutrition or some other metabolic factors.

MATERIAL and METHODS

Pineal bodies of camel were collected from cairo slaughter house. The pineal extract was prepared according to the method described by MILCU et al. (1963).

The experiments were done on four groups of newly born albino rats ten animals each; one group as control and the other three experimental. All rats were caged with their mothers.

Experimental animals:

All experimental animals were subjected to a daily intraperitoneal injection, starting from the 2nd day postnataly up to the 28th day with either:

Group I: 100 ugm Melatonin dissolved in 1ml dist. water
Group II: 100 ug Serotonin dissolved in 1ml dist. water.
Group III: 5.5 ml/Kgm body weight pineal extract (this dose corresponds to 2mg proteinic nitrogen which was calculated according to Kjeldahl method).

At the age of 4 weeks, each group of injected animals as well as the control group were weighted and compared using a sensitive balance. The animals were then used for tracer study.

Horseradish peroxidase (HRP) tracer study:

Five animals from each group including the control were subjected to tracer study. The animals were lightly anaesthetized with ether, the sciatic nerve was exposed through a small incision in the skin and muscles and fascia on the middle of the thigh. 10mg of HRP-(Sigma type II) dissolved in 10ml of saline, was repeatedly droped on to the nerve. After exposure to HRP for 60 min., the nerves were fixed by immersion in 2.5 purified glutaradhyde in phosphate buffer (pH 7.4) for 4 h. After overnight washing in phosphate buffer (PH 7.4); the sciatic nerves were cut into 0.2 mm. length under a dissecting microscope and the pieces were incubated in diamino-benzidine (GRAHAM & KARNOVSKY, 1966). The specimens of nerves were then processed as for electron microscopy i.e. postfixed in 2% osmium tetroxide, dehydrated in graded ethanols, passed through epoxy-propane and embedded in epon. Sections (1 um) were examined unstained.

RESULTS

1) Study of growth:

Data in table 1 & Fig. (1) shows that the mean weights of the injected groups was decreased below the mean control value and were all statistically highly significant ($P/ 0.001$).

Serotonin injected animals showed the lowest figures of weight with a mean of (14.1 $\pm$ 0.52) gm, followed by pineal extract which showed a mean of (16.3 $\pm$ 0.66) gm and melatonin (17.65 $\pm$ 0.78) mg, compared to control group which showed a mean weight value of (24.6 $\pm$ 0.75) gm.

2) Study of perineurial barrier function:

a) Control nerve (Fig. 1 a):

In the sciatic nerve of a control animal, HRP was observed in epineurial connective tissue, but it did not penetrate into the endoneurium being prevented by the perineurium, also, the reaction product did not leak through the endo neurial vessels which were filled with the reaction product.

b) Injected animals nerves:

It is shown from (Fig. 2a, 3a, 4a) that the distribution of the reaction product is exactly look like the control. No leakage of HRP either through the perineurium or endoneurial vessels was observed in the endoneurium of any of the nerves of injected animals whether with extract (Fig. 2a) or Serotonin (Fig. 3a) or melatonin (Fig. 4a).

DISCUSSION

The present study confirms previous observations that pineal extract and or its active substances serotonin and melatonin restrict somatic growth.

Regarding the mechanism for this delay, the idea that some pineal constituents diminished growth hormone synthesis or secretion was proposed. The wide variation in plasma GH levels so that, the concentration of GH in random blood samples was found to vary widely. As a result of pulsatile secretion (TAKAHASHI et al. 1971; DUNN et al. 1974 & RONNEKLEIV, 1975). This led to conflicting results concerning this subject.

In the present study we tried to investigate the problem from the begining. First the delay in growth in rats injected with extract or its active substances was very unlikely due to decrease in food intake (undernutrition) because pre and post-natal undernutrition induces an irreversible damage to the perineurial diffusion barrier of the rat sciatic nerve (SIMA and SOURANDER, 1973 & 1974; THOMAS and SOURANDER, 1977). This diffusion barrier has reached its physiological maturity at the age of 4 weeks, (KRISTENSSON and OLSSON 1971). The time we killed our rats was 4 weeks, so the damage of the perineurium produced by undernutrition must have been initiated before the diffusion barrier has reached its functional maturity. We started to inject rats at the age of 2 days, and the perineurium of all rats in this study showed a normal intact barrier in all experimental animals like the control. This means that the rats in the present study have not subjected to any sort of undernutrition.

The results of the present investigation confirms the fact that the pineal extract and its active substances restrict growth. The mechanism of this restriction is at least not due to undernutrition and is due to a metabolic factor which remains to be investigated in a well constructed and a stepwise experiments to avoid contradictory results.

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Table (1)

Weights of different group of animals after 4 weeks survival, a control group and three groups treated with either Melatonin, Pineal Extract or Serotonin daily injection for 22 days

Control gm n=10	Melatonin gm n=10	Extract gm n=10	Serotonin gm n=10
Mean = 24.6	17.65	16.3	14.1
SD = 2.37	2.47	2.08	1.63
SE = 0.75	0.78	0.66	0.52
Significance	(P/ 0.001)	(P/ 0.001)	(P/ 0.001)

LIST OF FIGURES

Fig. (1 a):

T.S. through the sciatic nerve of a control animal treated with peroxidase, unstained. Note the dark reaction product in the epineurium (ep) which serrounds the nerve fascicle. There is a sharp demarcation of the staining outside the perineurium (p). The endoneurium is otherwise unstained.

Fig. (2a, 3a, 4a):

T.S. through the sciatic nerves treated with peroxidase unstained for animals injected with either:

2a. Extract 3a. Seratonin 4a. Melatonin

Note that the dark reaction product has the same distribution in all the three spicimens like the control. Reaction product is also seen in the endoneurial vessels (large arrows), where red blood corpuscles have stained for endogenous peroxidase activity.

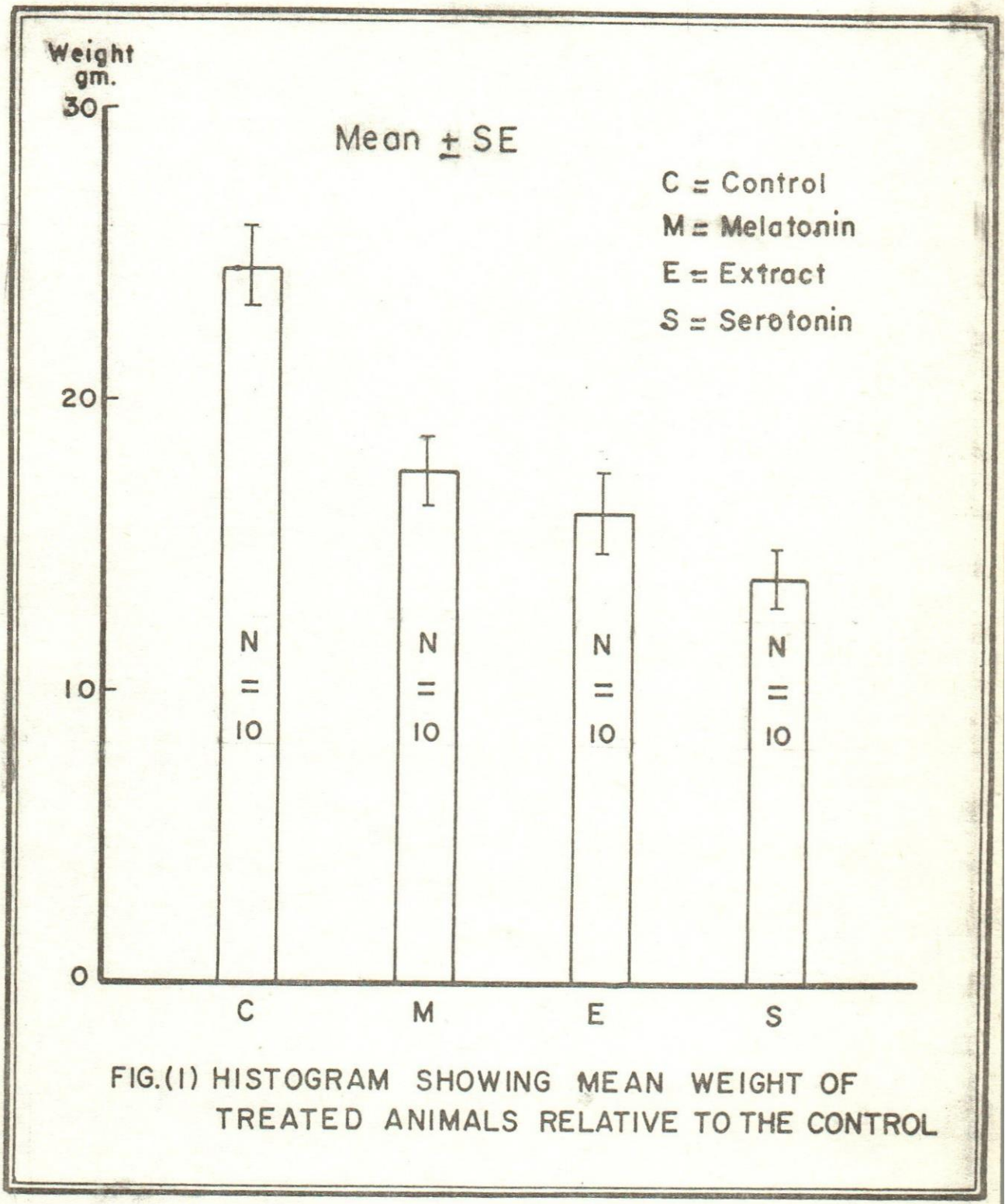
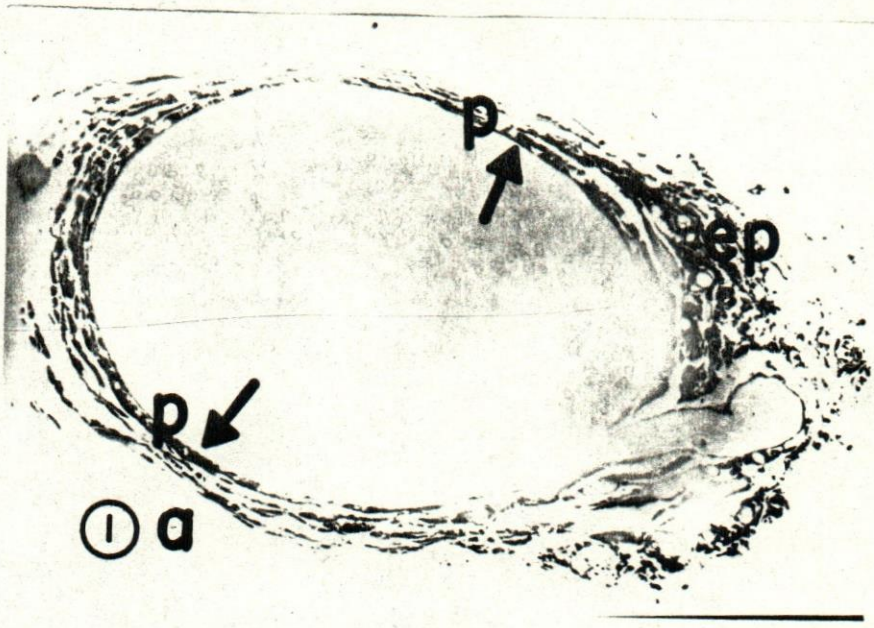


FIG.(1) HISTOGRAM SHOWING MEAN WEIGHT OF TREATED ANIMALS RELATIVE TO THE CONTROL



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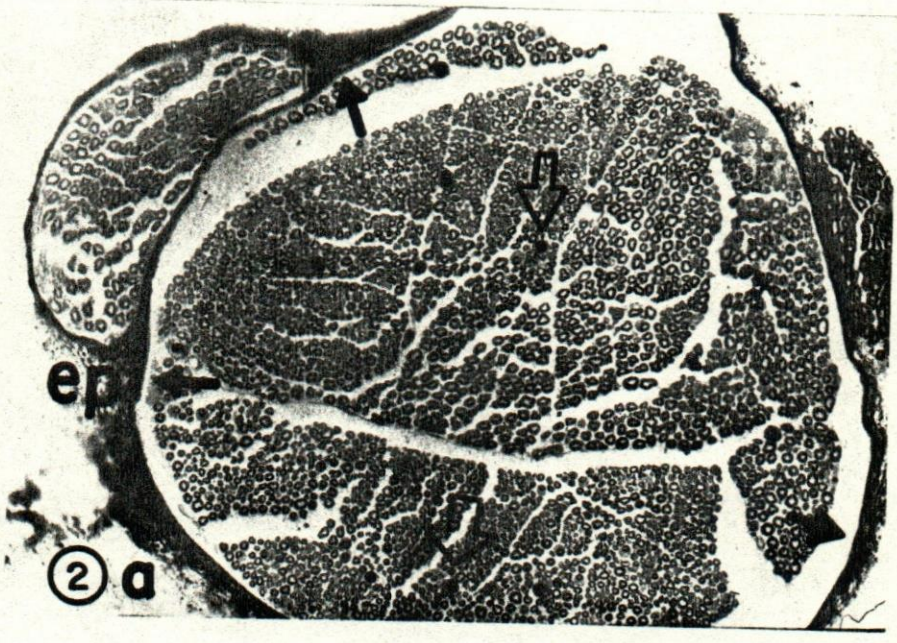

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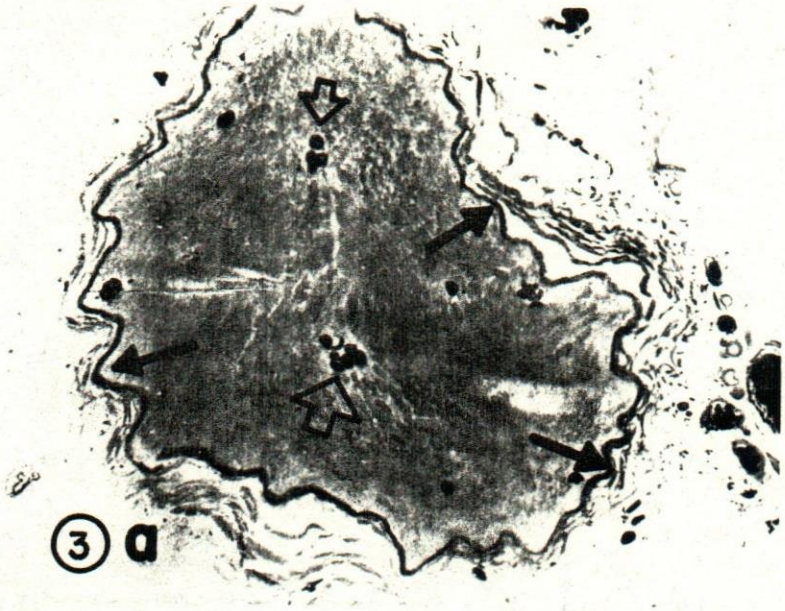
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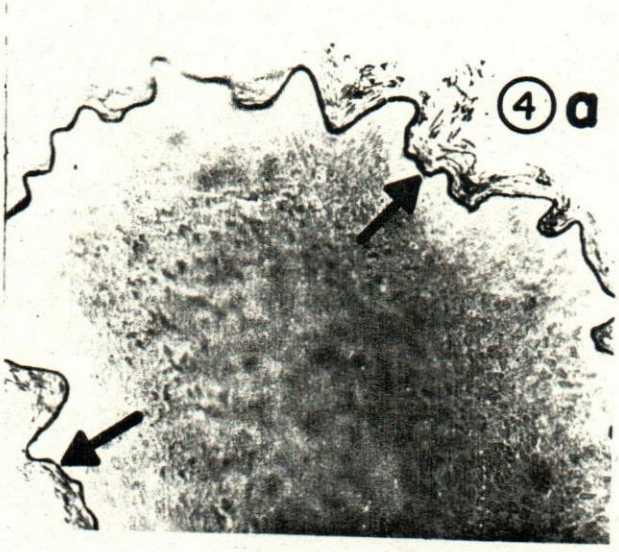
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