FOLKE RØNNIKE

THE PLANT HORMONE EFFECT OF SERUM FROM HUMANS AND SOME INDIVIDUAL SPECIES OF ANIMALS

With Special Consideration of the Effect of Serum from Women during Pregnancy and in Early Puerperium and also from Children

Det Kongelige Danske Videnskabernes Selskab

Biologiske Meddelelser **24**, 2

Kommissionær: Munksgaard

København 1967

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Selskabets sekretariat og postadresse: Dantes Plads 5, København V.

The address of the secretariate of the Academy is:

Det Kongelige Danske Videnskabernes Selskab,
Dantes Plads 5, København V, Denmark.

Selskabets kommissionær: MUNKSGAARD's Forlag, Prags Boulevard 47, København S.

The publications are sold by the agent of the Academy:

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Synopsis

1) Serum from human individuals, cattle, hens and pigs carries out an inhibitory effect on the growth of roots of wheat, *Triticum savitum Lam.*

The effect of serum from various species can be mutually different. The same is valid for the effect of serum from different breeds within the same species.

2) Serum from children in the 1/2–3 year-old age group acts less inhibitory than serum from adult subjects. This is confirmation of the Czech investigation with *Lupinus albus L.* in 1961.

Serum from younger subjects in cattle and hens has a less inhibitory effect than serum from adult individuals; on the other hand the opposite condition has been shown in pigs.

Thus it is not a general biological phenomenon that serum from non-adult subjects acts in a less inhibitory manner than serum from adult subjects.

3) In adult human individuals serum from early puerperal women has a less inhibitory effect than serum from non-pregnant women.

Individual sera of pregnant women acts less inhibitory than serum from non-pregnant women.

PRINTED IN DENMARK
BIANCO LUNOS BOGTRYKKERI A/S

CONTENTS

1. Introduction

a. On the plant hormone effect in human individuals in general. It is known that in human serum and urine there are to be found substances of a chemical structure that is partly identical with, and partly strongly similar to, genuine plant hormones.

However, the substances in the human and animal organism that have an influence on the metabolism of plants have been the subject of very infrequent investigations. A summary of these was given in the author's doctoral thesis (Rønnike 1961). In this work it was shown that the effect of human serum was due to substances that could be characterized as phytohormones or plant hormones. From this will be seen such organic substances, which at low concentrations promote, inhibit or qualitatively influence the growth of plants so that the effect is not dependent on the energy content of the substances or on their content of essential elements (Larsen 1955). Out of several hundred subjects the author found very few whose serum showed special strong phytohormone effect. This renders probable that these persons were physiologically identical with one of the described subjects of Kögl, Haagen Smit & Erxleben (1933) with specially strong urine-auxine-effect.

b. The plant hormone effect in children and individual species of animals. Čížková, Ulrychová & Růžička (1964) investigated the effect of serum from children (older than 9 months) and adolescents and found that serum from these subjects was less inhibitory on the growth of plant roots of *Lupinus albus L.* than serum from adult individuals. A similar difference in the effect of serum from children and adults has been confirmed in the present work (section 4). In addition a comparison has been made of the effect of the serum from non-adult and adult cows,

hens and pigs. In cows and hens the same difference between the two ages as in humans was found. On the other hand in pigs it was found that there was a more inhibitory effect with the non-adult subjects than with the adult.

c. *The plant hormone effect in pregnant women.* As early as 1,500 B.C. the Egyptians had described a plant hormone effect of the urine of pregnant women. By comparison of the Carlsberg VIII papyrus in Copenhagen with similar prescriptions found in the medical papyrus in Berlin, IVERSEN (1939) was able to give a summary of a translation in which the following sentence appears: "You shall put wheat and barley into purses of cloth, the woman shall pass her water on it every day . . . if both sprout she will give birth . . . if they do not sprout, she will not give birth . . ."

In his treatise IVERSEN discusses the channels through which these prescriptions were introduced into European popular medicine where they have remained well known until this very day. BAYON (1939) wrote a monograph on the existence of the phenomenon in European popular medicine. In Danish popular medicine the phenomenon was described by MØLLER (1940).

A number of authors have been occupied with the phytohormone effect of urine from pregnant women: to name a few, KÖGL et al. 1933, MANGER 1933, HOFFMANN 1934, BAK 1936, SCHWIND 1938, WILBERG 1944, TOBIAS 1950 and v. BRESKA 1951. Most of them thought that they were able to confirm that the urine of pregnant women had a different effect on the growth of plants than the urine of non-pregnant women. KÖGL et al., who, from a quantitative point of view, employed the best plant test (coleoptile curvature test) of the above investigators, found by an investigation of the urine of ten 8 to 9-month-pregnant women a plant hormone excretion of about three times that of non-pregnant.

In this present work (section 5) a comparison is undertaken of the effect of serum from non-pregnant, pregnant and early puerperal women. An inhibitory effect could be shown with certainty in the early-puerperal that could quantitatively be characterized as being similar to the effect in children. By investigation of separate sera a less inhibitory effect of serum from the pregnant subjects than from the non-pregnant could also be shown.

In the present series it was, however. with pooled sera from these two human categories not possible to show any difference.

The statistic symbols that are employed in this treatise are in accordance with SNEDECOR 1956.

2. Technique

a. Methods of measuring the plant root inhibitory activity in blood and urine, and blood and urine fractions respectively. In plant physiology a large number of standard methods are employed for the quantitative estimation of plant biological activity, LARSEN (1955). The methods used in this investigation are described in the present section. There are two preliminary growth methods, I and II, and then the procedure proper with the two growth-in-solution methods, A and B. The two preliminary growth methods, I and II, and the two growth-in-solution methods, A and B, can be used interchangeably, I with II and A with B.

The principle of the quantitative methods described here is fundamentally the very simple one of measuring the length increments from roots of *Triticum sativum Lam.*, variety "Starke".

b. Preliminary growth, method I. Figure 1 shows the uniform sowing of wheat grains on wet filter paper, guided by holes in an acryl sheet. In Figure 2 the filter sheet after sowing is seen being wound round a plastic cylinder measuring 26×7 cm. Figure 3 shows preliminary growth of wheat at 48 hours at 22°C between sheets of wet filter paper rolled on plastic cylinders standing in a container of water. All the cylinders of one experiment have been placed in the same polythene bag.

c. Preliminary growth, method II. Another method of preliminary growth of wheat plants appears in Figure 4, which shows wheat grains sown on specially-constructed 21×30×0.6 cm acryl sheets in which 90 depressions in the shape of spherical segments have been made. The diameter measured in the plane of the sheet is 1.5 cm. The depth of the depressions is 3–4 mm. Two acryl sheets are used together, one serving as the base and one as the cover, separated by two sheets of filter paper. A glass ball (8 mm in diameter) is placed in each corner depression to prevent the sheets from being pressed together too hard. The acryl sheets are held together by two rubber bands. The setting

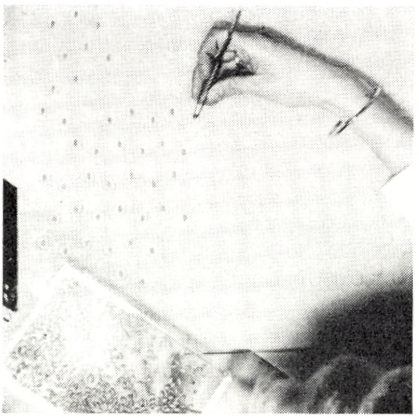

Fig. 1. Sowing of wheat grains.

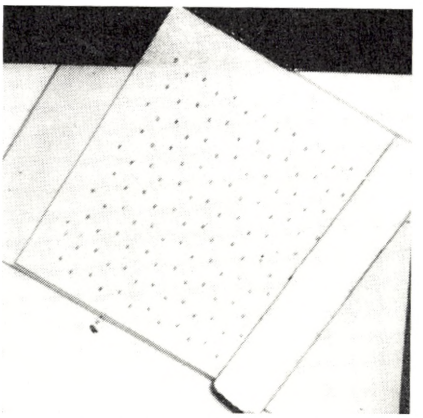

Fig. 2. Rolling a filter-paper sown with wheat round a plastic cylinder.

Fig. 3. Preliminary growth of wheat at 22° C (Figures 1–3: Method I of preliminary growth).

Fig. 4. Grains sown between acryl sheets (Method II of preliminary growth).

up of 15 pairs of sheets with about 1,300 sown grains, of which 35–50 per cent will prove usable for testing, takes $1^1/_2$ hours.

Figure 5 shows an air-cooling incubator used for the experiments. In the door of the incubator there is a built-in tube system that ensures forced circulation of air at constant temperature throughout the experiments, the cold air being sucked in from the refrigerating-plate compartment at the top of the incubator. A constancy of ± 0.2° C could be obtained. In the bottom compartment of the incubator a plastic container ($31 \times 32 \times 25$ cm)

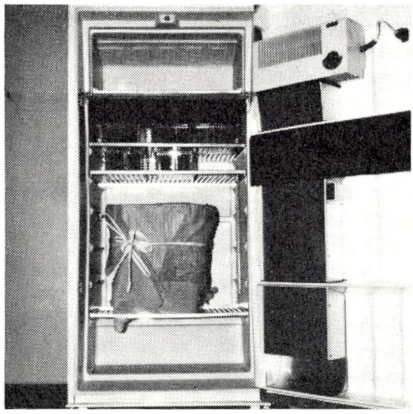

Fig. 5. Air cooling incubator for preliminary growth and growth in solution.

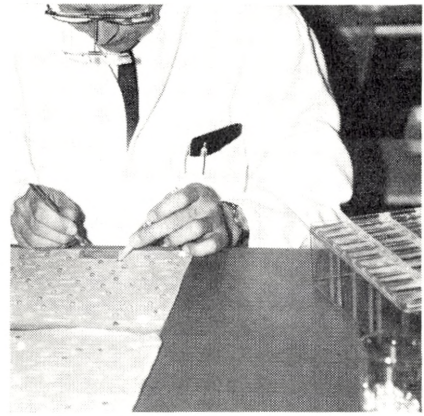

Fig. 6. Selection and measurement of plants before growth in solution.

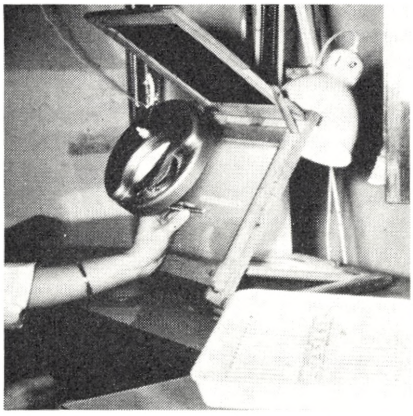

Fig. 7. A measuring setup.

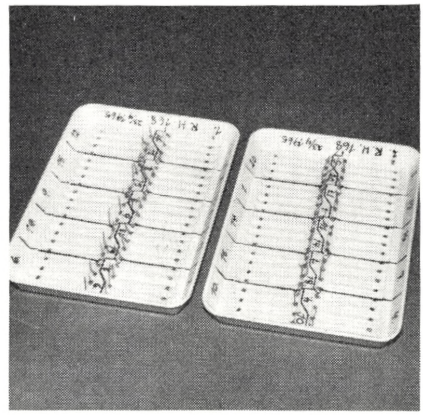

Fig. 8. Growth in polystyrol trays.

can be seen covered by a polythene sheet. The container holds up to 15 of the preliminary-growth sheet pairs. The water level is 2–3 cm above the bottom of the container. Only ion-exchanged + distilled water has been used. After 48 hours of preliminary growth at 22°C the plants have a length of 20 ± 10 mm.

d. The preparation and measurement of plants prior to, and after, growth in solutions. Figure 6 illustrates the selection and measurement of plants prior to growth in the solutions. During the handling of the plants it is advantageous to wear special magnifying glasses. A millimeter scale fitted on an angled knife

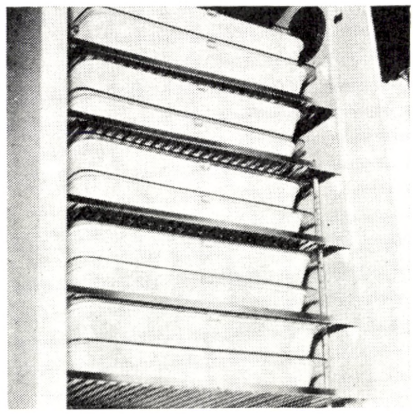

Fig. 9. A complete experiment of 20 solutions, each with 20 seedlings (Figures 8–9: Growth in solution, method A).

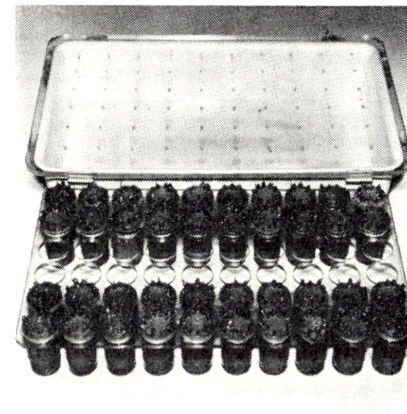

Fig. 10. Arrangement of 40 cups, each containing 10 plants (Growth in solution, method B).

is used for the measuring. Only the central positive geotropic of the seminal roots are used for the experiments, the lateral seminal plagiogeotropic roots are, in the main—see later—cut off. In the standard method only plants with a central root length of 20–22 (±5) mm are used. A 17×22×0.9 cm tray, divided into 40 sections of 4×2×0.9 cm, filled with re-distilled water may be used for preliminary floating of the plants before these are moved to the trays of method A (Figure 8) or the cups of method B (Figure 10)—see later.

Time will be saved if a dictating machine with a foot control switch is available for recording the measurements.

The final measurement of plants after growth in the solutions (after 24 hours in the standard method) is best done by means of a setup as shown in Figure 7: a millimeter scale behind a large lens.

e. Growth in solution, method A. On the right of Figure 7 can be seen one of the special polystyrol trays (obtainable from "Dansk Formstof", 7 Lergravsvej, Copenhagen S) of method A of the growth-in-solution methods, with the solutions to be tested. Five pairs of these trays cover a complete experiment. Figure 8 shows a pair of trays, i.e. a whole experiment in miniature, repeated five times in the aggregate experiment, where each pair of trays has its own random succession of the twenty different solutions of the experiment, i.e. a block experiment arrangement.

f. Growth in solution, method B. Figure 10 shows a setup consisting of 40 cylinder-shaped 25 ml cups measuring 5×2.5 cm (obtainable from the firm of "Nunc", Algade, Roskilde, Denmark). The growth-cups setup occupies far less space than the polystyrol-tray setup. The cups are discarded after use. In the plastic lids 10 holes of 3.6 mm diameter have been cut. The holes are peripheraly arranged so that the upper inner edge of the cups partially closes the holes, thus allowing access for the roots only and preventing the whole plants from falling into the liquid. Another type of lid (obtainable from "Helge Buchs Eftflg.", 55 Kapelvej, Copenhagen N.) is made of acryl plastic and has an inner diameter of 29 mm and a thickness of 4.5 mm, with 10 part-cylindrical, part-conical holes, 5×2.6 mm. This gives extremely good support for the seedlings and renders unnessary the time-consuming cutting off of the lateral seminal roots of the plants.

After use the lids are disinfected with a strong hydrochloric acid solution.

In the standard method 20 plants are grown in each solution, 10 in each of two cups. In many experiments, however, 30 or 40 plants have been used in each solution.

The cups are coded at the beginning of each experiment, and the succession of the cups during the experiment is a random one.

The centre compartment of the incubator of Figure 5 contains 5 plastic boxes of cups with plants of the growth-in-solution method B.

3. Variability in the Inhibitory Effect of Human Serum on Root Growth

a. The preparation of the serum and salt samples. The blood samples were usually taken in the morning. The samples were left for a few hours and then centrifuged, the serum was removed with a pipette. On many occasions the blood serum was kept undiluted for many days at −25°C. Such storage, as demonstrated below, had no effect on the obtained values. The serum samples were diluted mostly to 0.5 or 1 per cent with a salt solution of half the concentration used by Shive (1915), i.e. 2.5 mM $Ca(NO_3)_2$, 7.5 mM $MgSO_4$ and 9.0 mM KH_2PO_4, denoted in the

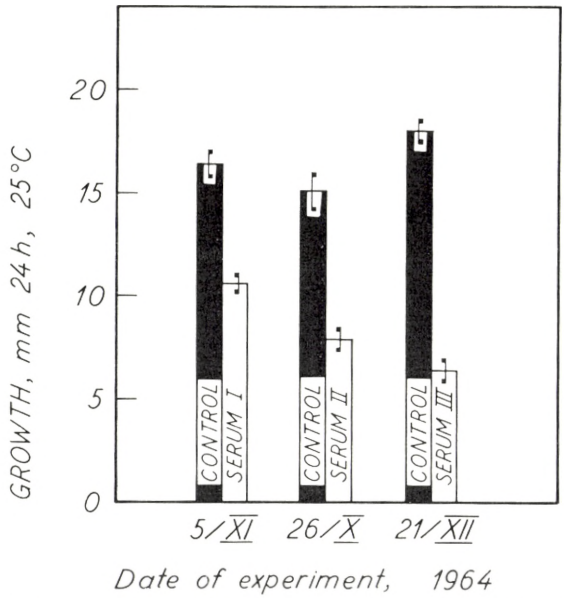

Fig. 11. Variability in the effect of three types of serum. Serum I, serum with a low inhibitory effect. Serum II, serum with an intermediate inhibitory effect. Serum III, serum with a high inhibitory effect. Growth in control (Shive's solution) and in serum diluted to 1 per cent with Shive's solution. Shown at the head of each column is the S.D.

following as "Shive". Sometimes, however, the concentrations used by SHIVE were used (the experiments in Figure 14). In some experiments a phosphate buffer solution 1/150 M at pH 6.0 with addition of 5 mM $Ca(NO_3)_2$ has been used. The serum dilutions were prepared immediately prior to use. Growth in salt solution without serum served as a control.

At first *Lupinus albus L.* was used by the author, but was abandoned in favour of *Triticum sativum Lam.* as relatively large quantities of serum—0.1 ml—were required for each Lupinus plant compared with about 12.5 μl for each Triticum plant.

b. The variability in the effect of one fresh serum sample within a single experiment. In experiments without addition of serum, standardized as described above, the S.D. of a single determination was 1–2 mm and with addition of serum to 1 per cent, 0.5–1.0 mm.

Figure 11 illustrates the variability in the effect of fresh serum samples in three different experiments. The experiments de-

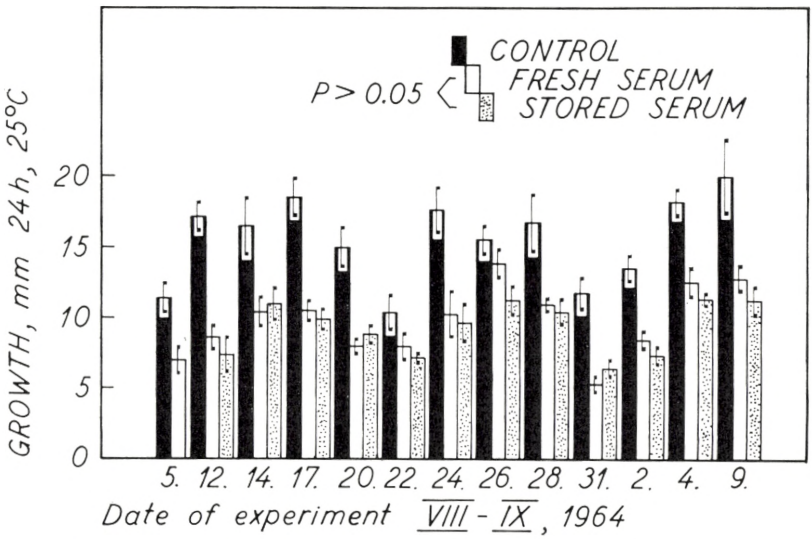

Fig. 12. Effect of storage of serum taken on a single day of one normal person. Comparison with the effect of non-stored serum in 13 experiments. Serum diluted to 1 per cent with Shive's solution. – First column in each experiment: Control growth, i.e. growth in Shive's solution. – Second column in each experiment: Growth in dilution of non-stored serum. – Third column in each experiment: Growth in dilution of serum stored from 5/VIII 1964. – Shown at the top of each column is $t_{.05}{}^{s}\bar{x}$. – Conclusion: No difference in inhibitory effect of stored and non-stored serum ($P > 0.5$).

monstrate the effect of sera with, respectively, a low, a medium and a high inhibitory effect. Each column value in Figure 11 is the mean of growth of 200 roots. The S.D. is shown at the head of each column.

c. The variability in the effect of non-stored serum compared with stored serum. The influence of serum is also demonstrated by the thirteen experiments of Figure 12. Serum from a single, normal person was used in each of the 13 experiments carried out on different days. On the first day of the experiments a large serum sample was prepared and pipetted off in 2 ml portions, these were kept at −25°C until use. In each experiment the effect of one stored portion was compared with freshly prepared serum. Figure 12 shows the variability of the mean growth in: (1) Control solution (Shive's solution), (2) 1 per cent diluted serum, prepared from samples of freshly drawn blood, and (3) 1 per cent diluted serum, prepared from serum stored at −25°C.

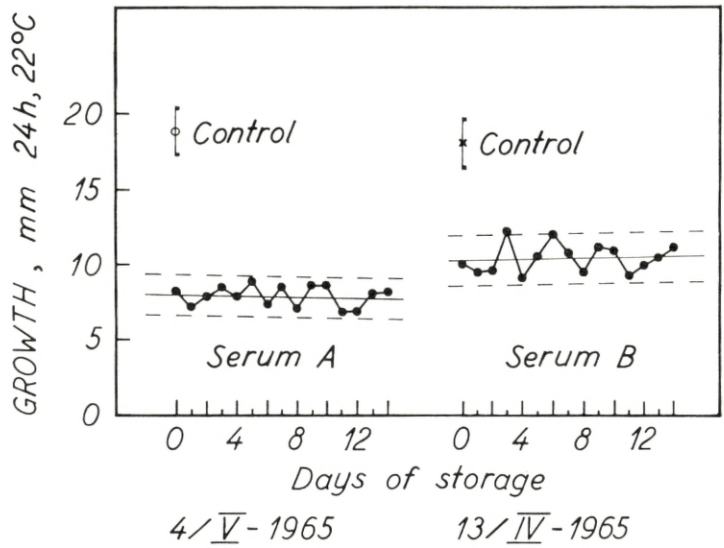

Fig. 13. Effect of storage of serum taken on many days from two persons, A and B. Two experiments: 4/V 1965, experiment with serum of person A and 13/IV 1965, experiment with serum of person B. Serum diluted to 1 per cent with Shive's solution. – Solid straight lines: Lines of the estimating equations for the two sets of data. Dashed lines: Zones of $\pm t_{.05} s_{Y.X.}$ – Conclusion: No significant regression ($P > 0.5$) in the two experiments, i.e. storage had no effect on the inhibitory effect of serum.

It is seen that although variations occur in the total growth, no significant difference could be demonstrated between the freshly prepared serum and the stored serum, ($P > 0.05$). Thus only small variations may have occurred in the inhibitory effect of the blood from the subject during the period of the sampling.

That storage at $-25°C$ does not alter the inhibitory effect is also demonstrated by the two experiments from two normal persons *A* and *B* shown in Figure 13. The serum samples from each person were each examined in a single experiment in such a way that all the determinations of the effect of all the serum samples of each person were carried out simultaneously. Serum prepared from a blood sample taken daily on 15 consecutive days and stored undiluted at $-25°C$ for up to 15 days is without influence on the inhibitory effect of serum from the two persons.

These experiments also demonstrate that only slight variations in the inhibitory effect of serum seem to occur in normal persons.

d. Influence of serum concentration, growth time, growth temperature and pooling of serum. The influence of variation of serum concentration, growth time, growth temperature and pooling of serum is described in section 6.

4. The Inhibitory Effect of Serum from Children, Chickens, Calves and Young Pigs compared with the Effect of Serum from Adult Individuals

a. The effect of serum from different species and breeds. The effect of serum from different species can show large variations. Thus in table 1 it can be seen how the effect of serum from pigs differs strongly from the effect of serum from the human individual and cattle.

The effect of serum from different breeds within the same species of animal can also vary, table 2. For instance, in hens it is possible to show a difference between White Leghorns and Brown Leghorns and between Brown Leghorns and White Plymouth Rocks, and in cattle between Red Danish Milk Breed and Black and White Danish Milk Breed.

b. The effect of serum from children and adults. Čížková, Ulrychová & Růžička (1964) have shown that serum from children has a less inhibitory effect than serum from adults. Čížková et al. used *Lupinus albus L.* In order to investigate whether it would be possible to demonstrate this difference with

Table 1. The Effect of Serum from Different Species.

SPECIES	No. of individuals	Growth, 24 hrs., 22° C $\pm t_{.05^8\bar{x}}$ mm
Man (*Homo sapiens*)	7	7.3 ± 0.4
Cattle (*Bos taurus L.*), Red Danish Milk Breed	6	8.3 ± 1.7
Pig (*Sus scrofa L.*), Danish Landrace	7	13.4 ± 1.3

Growth in serum diluted to 1 per cent with Shive's solution. One experiment: 18/I 1966.

TABLE 2. The Effect of Serum from Different Breeds.

BREEDS Date of experiment	No. of individuals	Growth, 24 hrs., 22° C $\pm t_{.05} s_{\bar{x}}$ mm	Probality of no difference
Hens (*Gallus domesticus L.*)			
White Leghorn	10	13.5 ± 0.4	$0.01 < P < 0.05$
Brown Leghorn	10	12.1 ± 0.8	
6/X 1965			
Hens (*Gallus domesticus L.*)			
Brown Leghorn	10	12.9 ± 1.2	$0.005 < P < 0.01$
White Plymouth Rocks ...	10	10.8 ± 0.9	
25/IX 1965			
Cattle (*Bos taurus L.*)			
Red Danish Milk Breed ...	10	12.3 ± 1.2	
Black and White Danish			$0.025 < P < 0.05$
Milk Breed	10	14.1 ± 1.2	
26/XI 1965			

Growth in serum diluted to 1 per cent with Shive's solution. Three experiments.

other plant species the experiments were carried out with *Triticum sativum Lam.*

The results are shown in Figure 14; in each of the 15 experiments an equal number of children and adults took part, in all 72 children and 72 adults.

Figure 14 shows that the plant roots grew longest in the control solution, next longest in the serum from children and least in the serum from adults ($P < 0.001$). Thus serum from young children inhibits the growth of the cells in the meristems near the tips of the plant roots less than serum from adult individuals, and so the experiments confirm the results of ČÍŽKOVÁ et al.

It should be pointed out that the experiments in Figure 14 were carried out with a technique that varies slightly from that described in section 2 as the experiments were carried out in a period prior to the complete standardization of the technique.

ČÍŽKOVÁ et al. also found that the inhibitory effect increased with age, so that the effect at 15–18 years of age corresponded to that of adults.

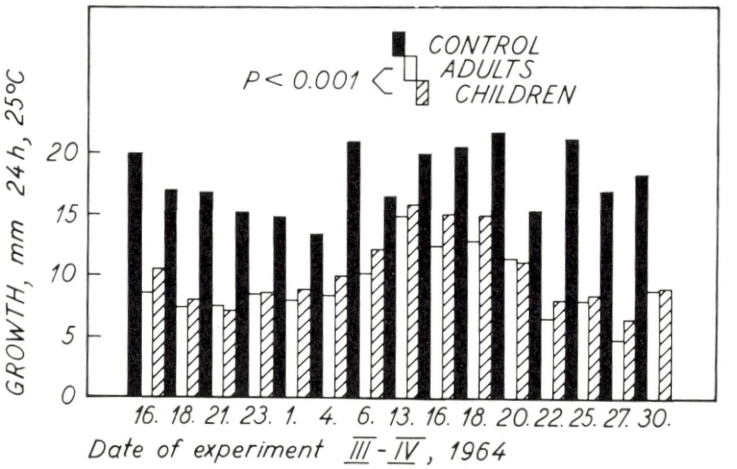

Fig. 14. Effect of serum from children and adults. 15 experiments with serum from 72 different normal children $^1/_2$–3 years old and 72 different normal adults. – First column in each experiment: Growth in control (Shive's solution). – Second column in each experiment: Average growth in 1 per cent dilutions of serum from 5 (4–5) different normal adults. – Third column in each experiment: Average growth in 1 per cent dilutions of serum from 5 (4–5) different children. – Conclusion: Lesser degree of inhibition of serum from children compared with serum of adults ($P < 0.001$).

Comparative experiments with serum from children of various ages and adults are illustrated in Figure 15. It can be seen how the difference in the effect of serum from children and serum from adults is clearly marked in the two experiments with children under the age of 2. On the other hand a significant difference could only be demonstrated in one of the two experiments with children in the 6–8 age group, and no difference could be shown in either of the two experiments with children aged about 15. The results of these experiments are in agreement with those of Čížková et al.

c. The effect of serum from non-adult and adult cattle and hens. In order to see whether a difference comparable with that that exists between children and adults could also be shown in animals, determinations of serum from hens, cattle and pigs were carried out.

The effect of serum from hens of the White Plymouth Rocks breed can be seen in Figure 16. As a control in each experiment a number of about-one-year-old hens were used. In the experiments the effect of serum from the hens was compared with

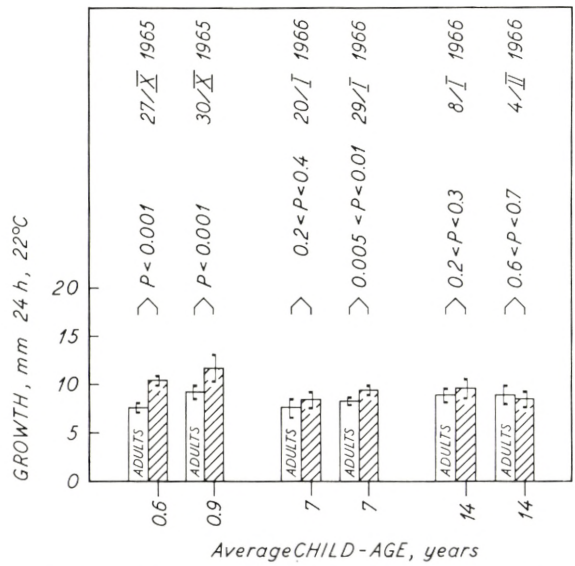

Fig. 15. Effect of serum from children at different ages. 6 experiments with serum of 50 different normal children at different ages and serum of 46 different normal adults. Serum diluted to 1 per cent with Shive's solution. – First column in each experiment: Average growth in dilutions of serum from about 10 adults. – Second column in each experiment: Average growth in dilutions of serum from about 10 children. Shown at the head of each column is $t_{.05} s_{\bar{x}}$. – Conclusion: Only at the two youngest ages did all the children have inhibitory serum effects different from adults.

serum from 17-, 69-, and 125-day-old chickens. The blood from the 17-day-old chickens was obtained by decapitation, whilst the blood from the others was taken from a wing vein.

A clear difference could be shown in all three experiments ($P < 0.001$, $P < 0.001$ and $0.01 < P < 0.025$).

The inhibitory effect of serum from cattle is illustrated by the last three experiments in Figure 16. A comparison of the effect of serum from 3-week-old calves and 24-week-old heifers was made with the effect of serum from adult cattle. In both cases a difference could be shown between the younger animal and the adult ($P < 0.001$). On the other hand it was not possible to show a difference between the effect from calves and the effect from heifers—the last experiment in Figure 16.

In respect to hens and cattle the same relation as in man could thus be shown: a less inhibitory effect in the non-adult subjects compared with the effect from adult.

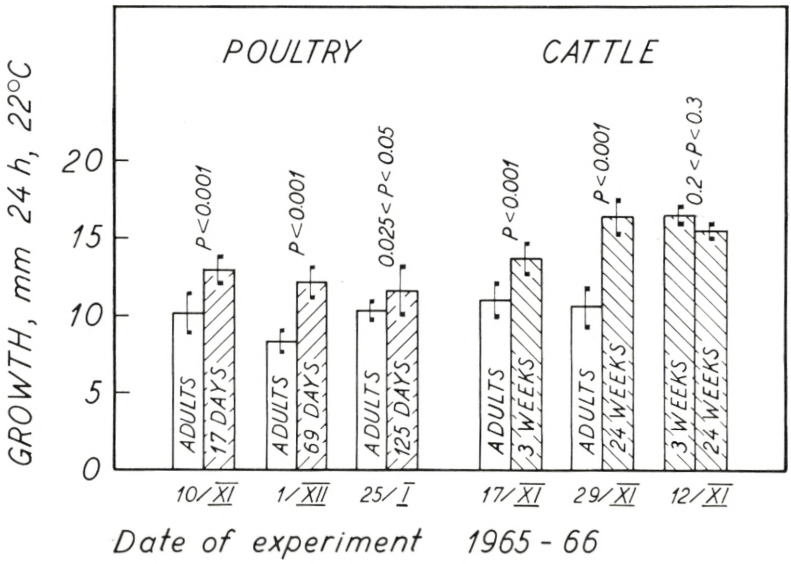

Fig. 16. Effect of serum from hens (White Plymouth Rocks) and cattle (Red Danish Milk Breed) at different ages. 6 experiments showing effect of dilutions to 1 per cent of serum from 118 different animals. – First column in each experiment: Average growth in serum dilutions of about 10 adult animals (except experiment 12/XI 1965). – Second column in each experiment: Average growth in serum dilutions of about 10 non-adult animals. – At the head of each column is shown $t_{.05}s_{\bar{x}}$. – Conclusion: Lesser inhibitory effect of serum from non-adult animals of hens and cattle compared with the effect of serum from adult individuals.

d. The effect of serum from non-adult and adult pigs. In table 3 the inhibitory effect of serum from adult pigs is reproduced compared with the effect of serum from five-week-old and six-

TABLE 3. The Effect of Serum from Pigs at Different Ages.

AVERAGE AGE Pigs (*Sus* scrofa *L.*) Danish Landrace Date of experiment	No. of individuals	Growth, 24 hrs., 22° C $\pm t_{.05}s_{\bar{x}}$ mm	Probability of no difference
3–5 weeks	9	12.7 ± 1.5	
3 years	10	15.5 ± 0.3	$0.001 < P < 0.005$
3/XII 1965			
6 month	10	14.9 ± 1.2	
3 years	10	17.2 ± 0.9	$0.005 < P < 0.01$
9/XII 1965			

Growth in serum diluted to 1 per cent with Shive's solution. Two experiments.

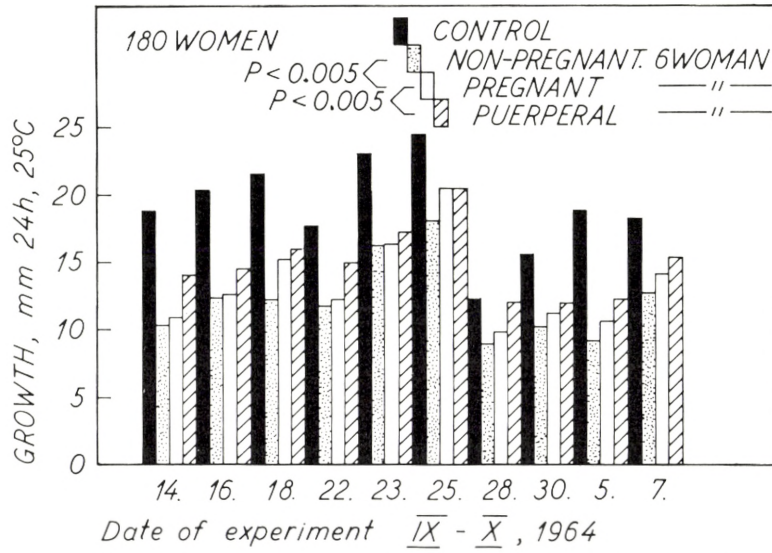

Fig. 17. Effect of serum from non-pregnant, pregnant and puerperal women. 10 experiments with growth of about 4000 plants in dilutions to 1 per cent of serum from 180 women. – First column in each experiment: Growth in control solution (Shive's solution). – Second column in each experiment: Average growth in dilutions of serum from 6 different, non-pregnant, normal women. – Third column in each experiment: Average growth in dilutions of serum from 6 different women, normal pregnant in the third trimester. – Fourth column in each experiment: Average growth in dilutions of serum from 6 different women in normal, early puerperium. – Conclusion: Differences between effect of serum of the three categories of women ($P < 0.005$).

month-old pigs. It can be seen that in pigs a stronger inhibitory effect of the serum from younger animals could be demonstrated compared with the effect from adult animals: that is to say, the inverse relation to that which obtains in man and the two other investigated species of animal.

Thus it appears not to be a general phenomenon that the inhibitory effect of the serum from adult subjects is greater than the effect from non-adult subjects.

5. The Inhibitory Effect of Individual Serum Samples from Normal Pregnant and Puerperal Women

a. The effect of serum from non-pregnant, pregnant and puerperal women. The effect of serum from 60 non-pregnant, 60 pregnant and 60 early-puerperal normal women was investigated in 10 mutually independent experiments (Figure 17). In each

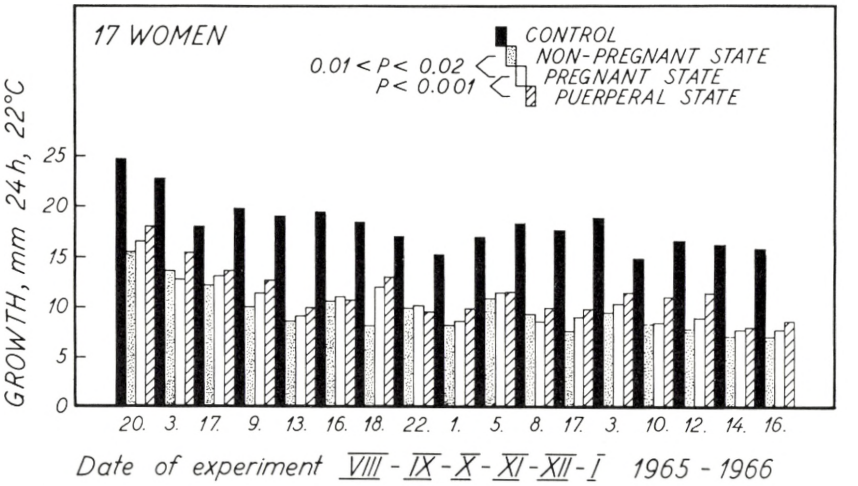

Fig. 18. Effect of serum from 17 different women during and after normal pregnancy. 17 experiments with growth of about 6000 plants. Each experiment shows the effect of serum from a single women in the pregnant and early puerperal state and 1 month or later after delivery. – First column in each experiment: Growth in control solution (Shive's solution). – Second column in each experiment: Average growth in dilutions to 1 per cent of serum from 6 (5–7) different days 1 month and/or later after delivery. – Third column in each experiment: Average growth in dilutions to 1 per cent of serum from 6 (5–7) different days during late pregnancy. – Fourth column in each experiment: Average growth in dilutions to 1 per cent of serum from 6 (5–7) different days in early puerperium. – Conclusion: Differences between effect of serum of the three categories of physiological states ($0.01 < P < 0.02$ and $P < 0.001$).

experiment an average of separate determinations on 6 non-pregnant, 6 pregnant (in the third trimester) and 6 early puerperal women was carried out. It can be seen from Figure 17 that the largest inhibitory effect could be shown from the non-pregnant, the least from the early puerperal and an intermediary effect from the serum from pregnant women. By calculations that include the separate experiments only it will often not be possible to show any difference, on the other hand by calculations that include all mean values of the three categories of women a significant difference can be shown with the significant figure as quoted on the figure.

b. The effect of serum from 17 women during and after pregnancy. The effect of serum during pregnancy, in early puerperium and one month or more after delivery was investigated in 17 normal women (Figure 18). Separate determinations were made of 18 serum samples from each woman: these were 6 samples

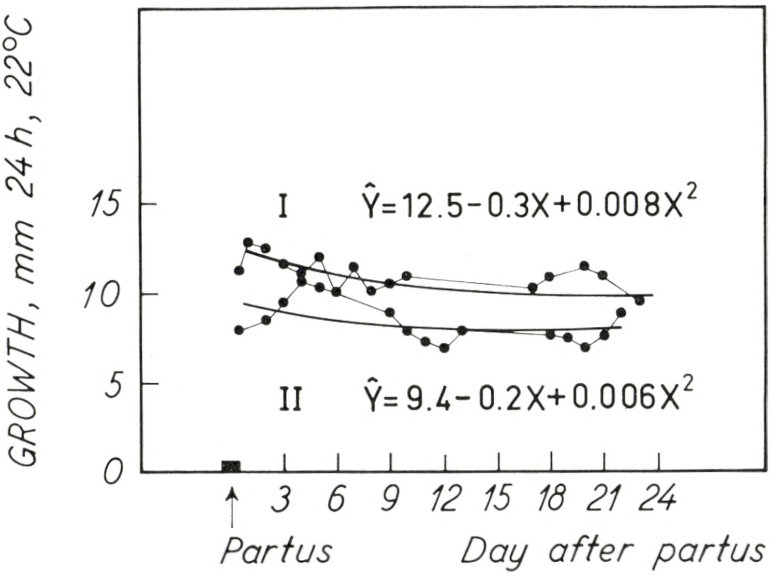

Fig. 19. Effect of serum of two women I and II in three normal puerperal weeks after delivery. Solid curved lines: Estimated parabolas of two sets of data. – Conclusion: Puerperal effect of less inhibition disappeared three weeks after delivery.

taken on 6 different days during the later pregnancy, 6 samples taken on 6 days in the early puerperium and 6 samples from 6 days at least one month after delivery. In this series of experiments it also could be shown that on an average the strongest inhibitory effect occurs in the non-pregnant, an intermediary effect in the pregnant and the weakest inhibitory effect in the early puerperium.

c. The effect of serum from two puerperal women in the three weeks after delivery. The effect of serum in the first three weeks after delivery from two women, *I* and *II*, is shown in Figure 19. Two second degree polynomials are shown in the figure: the graphical representations of these take into account the experimental data so that the sum of the square of the distances from the parabolas to the experimentally-found points is a minimum. The possibility that these curves are curvilinear is over 99 per cent and as constant level (where $dy/dx = 0$) is obtained after three weeks there is thus evidence that a lesser inhibition exists in the period just after delivery, and also evidence that this reduction disappears after three weeks. This experiment is thus in agreement with the average results of the previously described experiments.

6. The Inhibitory Effect of Pooled Serum

a. Pooled serum samples compared with individual serum samples. Individual determinations with serum from different individuals often show a large variation caused partly by analysis error and partly by the individual variation. This complicates a comparison of the effect of serum from different subject categories. In a comparison of the effect of serum from non-pregnant, pregnant and puerperal women it is thus necessary either to investigate serum from a very large number of subjects or, if fewer subjects are available, to investigate each subject repeatedly.

In order to investigate if the number of analyses could be reduced by employing pooled serum, a comparison was made of the growth in 1 per cent dilutions of individual sera and 1 per cent dilutions of a pooled serum produced from the same sera. The result of this comparison appears in Figure 20 where in two experiments a determination was made of the effect of serum from 20 women: that is 10 non-pregnant women in the experiment on 10/II 1966 and 10 early puerperal women in the experiment on 29/I 1966.

It can be seen that it has not been possible to demonstrate a difference between the average effect of the individual sera and that of the pooled serum. As expected it can also be seen that the standard deviation is less for the pooled serum than for the individual sera.

The experiments thus appear to show that pooled serum ought to be preferred for a comparison in groups of several subjects based on the greater precision with which it is possible to determine the average values and because fewer of the time-consuming plant-experiments need be made.

b. The effect of pooled serum from non-pregnant, pregnant and puerperal women. In a series of experiments a comparison was made of pooled sera from 60 non-pregnant, 60 pregnant and 60 early-puerperal normal women. Here a difference could be shown between the serum of the early-puerperal women compared with the serum of the other two categories: on the other hand no difference could be demonstrated in the present series between the effect of the serum from the pregnant women and the non-pregnant women.

Reproduced in table 4 are the results from one of the experiments in this series. Serum from the puerperal women had the

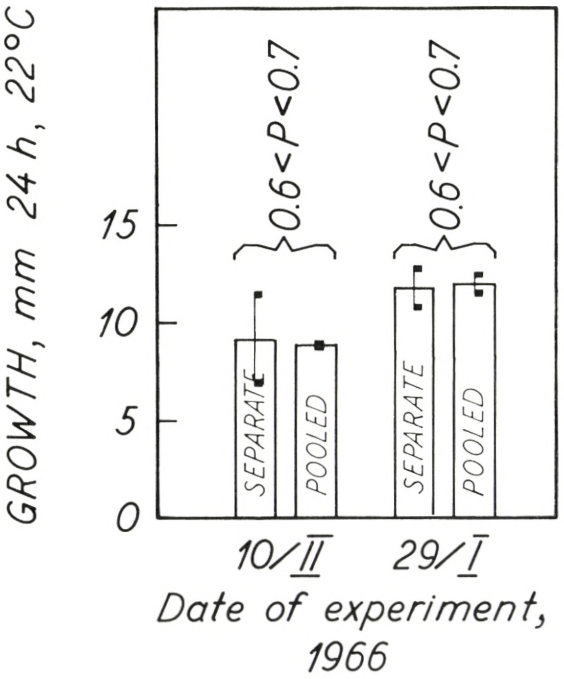

Fig. 20. Pooled serum compared with samples of individual serum. Two experiments: 10/II 1966: Serum from ten normal, non-pregnant women and 29/I 1966: Serum from ten normal, puerperal women. Serum diluted to 1 per cent with Shive's solution. At the head of each column is shown ± S.D. – Conclusion: No difference of average growth in two types of serum dilutions, lesser S.D. in pooled serum dilutions.

least inhibitory effect, 11.5 ± 0.4 mm, compared with 8.9 ± 0.6 mm in the pooled serum of the pregnant women and 8.7 ± 0.3 mm in the pooled serum of the non-pregnant women.

c. The effect of variation of concentration of pooled serum from puerperal and non-pregnant women. The effect of different concentrations of the pooled serum from 0.25 to 5.0 per cent from puerperal and non-pregnant women is shown in Figure 21. Also shown in this figure is the effect of 5 mM Ca^{++} and 10^{-6} M of the antiauxin para-chloro-isophenoxy-butyric acid (PCIB) at the same serum concentrations. The serum is diluted with a phosphate buffer 1/150 M at pH 6.0. After dilution adjustment is made with 0.1 N HCl to pH 6.0. It can be seen that an increase in the concentration of the serum alone does not cause any large increase in the inhibition. The difference between the two types

TABLE 4. The Effect of Pooled Serum from Puerperal, Pregnant and non-Pregnant, Normal Women.

TYPE OF POOLED SERUM (No. of determinations)	Average growth of 20 plants 24 hrs., 22° C $\pm$ t. $_{05}s_{\bar{x}}$ mm	Average growth of 120–140 plants $\pm$ t. $_{05}s_{\bar{x}}$ mm
Pooled serum of 60 puerperal women (6)	11.9 $\pm$ 1.1 11.9 $\pm$ 0.9 11.6 $\pm$ 1.4 11.4 $\pm$ 0.8 11.4 $\pm$ 1.3 10.8 $\pm$ 1.0	11.5 $\pm$ 0.4
Pooled serum of 60 pregnant women (6)	9.4 $\pm$ 1.5 9.4 $\pm$ 0.9 9.2 $\pm$ 1.0 9.1 $\pm$ 1.0 8.5 $\pm$ 0.9 8.0 $\pm$ 0.8	8.9 $\pm$ 0.6
Pooled serum of 60 non-pregnant women (7)	9.2 $\pm$ 0.8 9.1 $\pm$ 0.6 9.0 $\pm$ 0.8 8.7 $\pm$ 0.7 8.5 $\pm$ 0.9 8.4 $\pm$ 0.7 8.3 $\pm$ 1.0	8.7 $\pm$ 0.3

Pooled serum diluted to 1 per cent with Shive's solution. One experiment: 24/X 1966.

of serum is also small. The addition of 5 mM Ca^{++} markedly changes the effect of the two sera so that a distinct correlation between the concentration of the serum and its inhibitory effect is demonstrable. This becomes more distinct on the addition of both 5 mM Ca^{++} and 10^{-6} M PCIB. Here the growth increases strongly without the addition of serum. At the highest serum concentration a stronger inhibitory effect is obtained by the addition of Ca^{++} and PCIB than without the addition of Ca^{++}. For all the curves in Figure 21 it may be said that the pooled serum from puerperal women has shown less inhibitory effect than pooled serum from non-pregnant.

Reproduced in Figure 22 is the effect of a variation of two

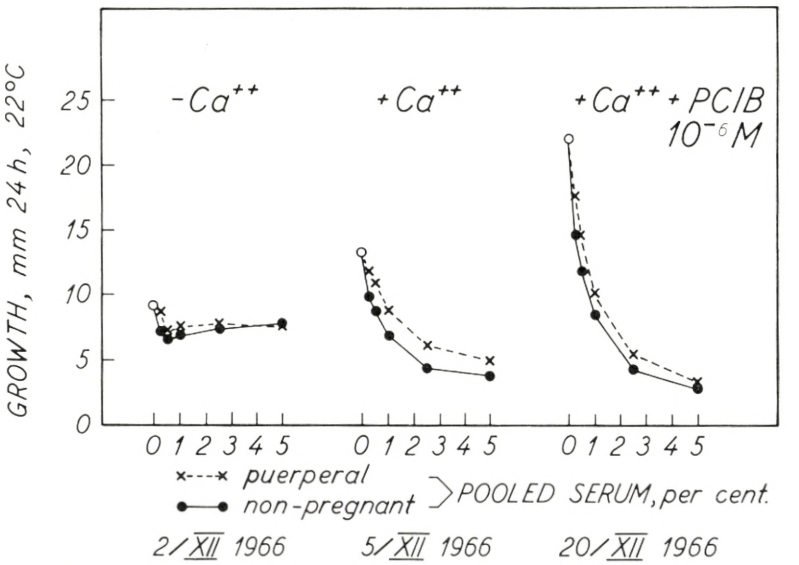

Fig. 21. Effect of different concentrations of serum without and with addition of 5 mM $Ca(NO_3)_2$ and 10^{-6} M of the antiauxin PCIB. Dilution of pooled serum with 1/150 M phosphate buffer at pH 6.0 to 0.25, 0.5, 1.0, 2.5 and 5.0 per cent. Three independent experiments. – Conclusion: Lesser puerperal inhibitory effect present in all cases of changed growth of plants.

PCIB concentrations for two different concentrations of pooled serum from puerperal and non-pregnant women: 0.5 and 5.0 per cent. The serum is diluted with Sørensen's phosphate buffer 1/150 M + 5 mM $Ca(NO_3)_2$ at pH 6.0. It can be seen that an increase of the concentration of PCIB to 10^{-5} causes a further growth of the roots of the plants used in the experiments; on the other hand an increase of the concentration to 10^{-4} results in normal growth. The effect of the serum is seen to be reduced in that no inhibitory effect of the serum is obtained on dilution to 0.5 per cent and the inhibition by dilution to 5.0 per cent is reduced. The serum from the puerperal women in relation to that from the non-pregnant is seen in all three curves to have the less inhibitory effect.

To sum up it may be said that despite the growth variation on addition of Ca^{++} and PCIB pooled serum from the puerperal shows a less inhibitory effect compared with serum from the non-pregnant women.

d. The effect of variation of pH-value. The effect of a variation of pH in pooled puerperal and pooled non-pregnant serum

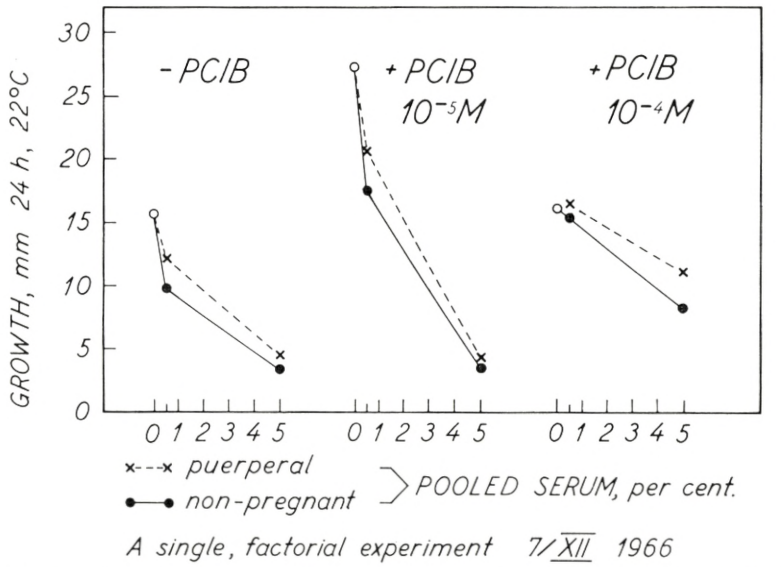

Fig. 22. Effect of two concentrations of serum at two concentrations (10^{-5} and 10^{-4} M) of the antiauxin PCIB. A single, factorial experiment. Dilution of pooled serum with 1/150 M phosphate buffer at pH 6.0 and 5 mM $Ca(NO_3)_2$ to 0.5 and 5.0 per cent. – Conclusion: Lesser puerperal inhibitory effect present in all cases of changed growth of plants.

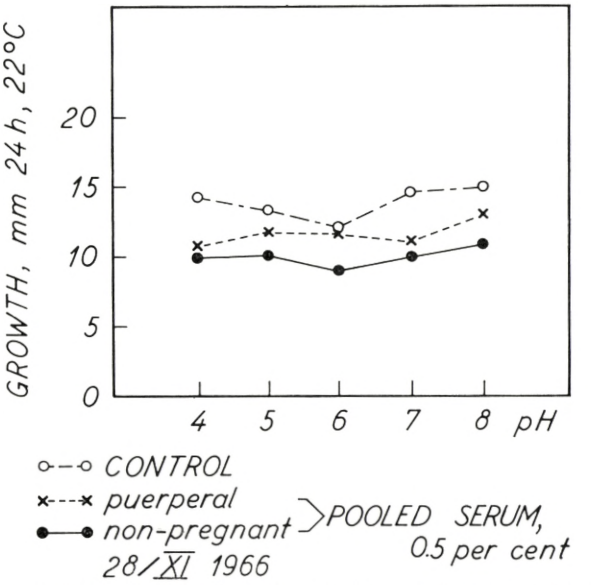

Fig. 23. Effect of different pH-values. Growth in pooled serum diluted to 0.5 per cent with 1/150 M phosphate buffer and 5 mM $Ca(NO_3)_2$. – Conclusion: Lesser puerperal inhibitory effect present at all pH-values.

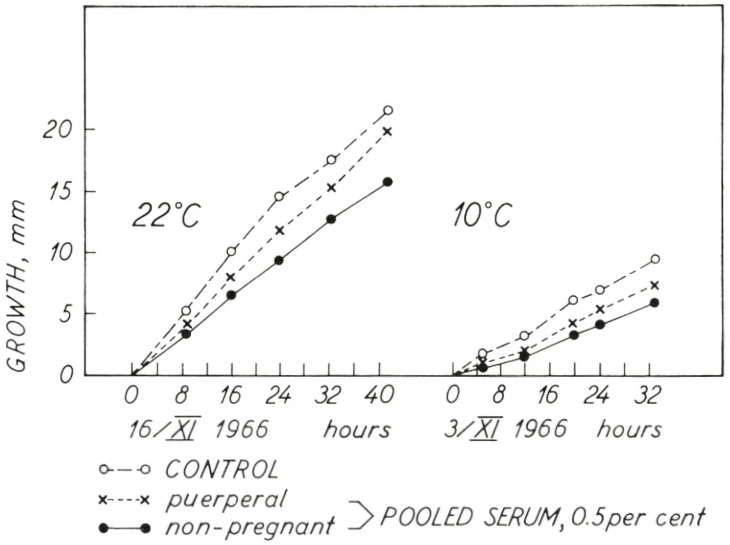

Fig. 24. Effect of different durations of growth at two temperatures, 10° C and 22° C. Serum diluted to 0.5 per cent with 1/150 M phosphate buffer at pH 6.0 and 5 mM $Ca(NO_3)_2$. – Conclusion: Lesser inhibitory puerperal effect present at both temperatures and at all times of growth.

diluted to 0.5 per cent is seen in Figure 23. Serum from the two categories of women is diluted with 5 mM $Ca(NO_3)_2$ in a phosphate buffer, 1/150 M at pH 6.0, as an outlet thinner. After this the adjustment of the pH values to pH 4.0, 5.0, 6.0, 7.0 and 8.0 is made by the addition of 0.1 N HCl or NaOH. Figure 23 also shows the larger growth in the dilutions with pooled puerperal serum than with pooled non-pregnant serum for the investigated pH values.

e. The effect of growth time and growth temperature. Figure 24 shows two experiments with growth in 0.5 per cent pooled serum from puerperal and non-pregnant women. The growth of the plants took place at respectively 10° and 22° C for up to 43 hours. On the whole proportionality between the growth and the length of the experiment could be demonstrated. Thus, in the serum solutions, there is in fact an actual inhibition of growth and not, for example, a delayed commencement of growth. As expected the quicker growth took place at 22° C.

In these experiments as well the serum from puerperal women had the less inhibitory effect.

7. Discussion

The discovered less inhibitory effect of serum from children in relation to adults is confirmation of the results of the Czech investigations made in 1961 (Čížková, Ulrychová & Růžička). *Lupinus albus L.* was used in these Czech investigations, whilst in the present investigations *Triticum sativum Lam.* was used. Thus, that it has been possible to show the same phenomenon with two different species of plants must be considered as additional confirmation of the existence of the phenomenon.

A similar relation as that in human individuals has, as mentioned, also now been shown in cattle and hens. On the other hand serum from non-adult pigs had a larger inhibitory effect than serum from adult pigs. In human individuals it has also been possible to show a less inhibitory effect in puerperal women in the first days after delivery compared with other normal adult persons. On the other hand it must be considered as uncertain if there is a difference in the inhibitory effect of serum from pregnant women compared with non-pregnant women.

The root inhibitory effect of human serum is determined by substances that must be characterized as phytohormones of predominently auxin type. These substances occur partly in the free state and partly bound to the serum proteins (Rønnike 1961). A dominating part of the inhibitory effect of urine and serum is due in all probability to indole-3-acetic acid, but it is also conceivable that other substances are playing a role as an antagonistic effect between various substances can also appear. An antagonistic effect can thus appear partly in the form of greater or lesser inactivation by binding, partly thereby that perhaps substances with a growth promoting effect may be found.

The difference in the effect of individual and pooled sera from pregnant women where the inhibition of the individual sera showed disparity in effect opposite the pooled sera, can thus possibly find an explanation in that the individual sera with the most strong inhibition contains either a deficit of growth promoting substances or a deficit of inactivating substances or both.

The experiments available up to now do not permit however a definite decision on whether there exists a qualitative difference as an explanation of the various serum effects and until further

it is quite natural to assume that the variation in the concentration of the same substances in various subjects is the most important factor. An experimental support of this is found in the experiments in section 6 with Ca^{++} and the antiauxin para-chlorophenoxybutyric acid (PCIB). Sufficiently thorough analyses of the results of the experiments with serum, Ca^{++} and PCIB are difficult to carry aut, but it should be stated that the antagonism between Ca^{++} and PCIB opposite pooled puerperal and pooled non-pregnant serum was fairly equally pronounced opposite both the two concerned types of serum at all the investigated concentrations of serum (Figures 21 and 22). In other experiments, too, it has not been possible to find a combination of serum, Ca^{++} and PCIB concentrations where the difference between the two serum types was diminished or augmented.

Thus, these relationships make it probable that the difference in the effect of serum from puerperal and non-pregnant women is more of a quantitative type than a qualitative one.

The less inhibitory effect in young subjects has as well as in human individuals also been shown in cattle and hens. No investigations exist that can show if the inhibitory effect in these species of animals in a qualitative respect differs from that in the human individual. It does not seem however to be a general biological phenomenon that serum from non-adult subjects works less inhibitory that serum from adult subjects, which the determinations on non-adult pigs seem to show as the effect here, as mentioned, was found to be more inhibitory than the effect in adult pigs.

It is uncertain as to the reason for this discrepancy between the species. Many considerations of a similar nature to those above about the interplay between inhibitory and promoting substances can be mentioned, to which also can be added considerations of different selectivity in the excretory activities in the various species of animals.

8. Acknowledgments

This work has been supported by grants from "Statens almindelige Videnskabsfond", "Fonden til Lægevidenskabens Fremme" and "Fondet for Dansk-Svensk Samarbejde".

The author is also indepted to each of the following: Professor, Dr. D. TROLLE and Dr. B. SØRENSEN for providing me with the necessary facilities at Rigshospitalet, Copenhagen; Professor, Dr. H. BURSTRÖM, Lund, for help with many plant-physiological problems; Professor, Dr. A. NEIMANN-SØRENSEN, Professor JOH. BÆLUM and Professor, Dr. HJ. CLAUSEN for help in supplying the necessary blood specimens of cattle, hens and pigs from The Royal Danish Veterinary and Agriculture College and The National Research Institute of Animal Science; Dr. MARIE ULRYCHOVÁ, Prague, for collaboration with some of the experiments; Dr. AXEL BIERRING and the many leaders of homes for children in Copenhagen for help in supplying blood samples of children.

Last, but certainly not least, I would like to express my sincere gratitude to the Director of Research at the Danish Institute of Protein Chemistry, mag. scient. KAY BRUNFELDT, for his critical examination of the manuscript.

9. Summary

(1) Serum from human individuals, cattle, hens and pigs carries out an inhibitory effect on the growth of roots of wheat, *Triticum sativum Lam.*

The effect of serum from various species can be mutually different. The same is valid for the effect of serum from different breeds within the same species.

(2) Serum from children in the $^1/_2$–3 year-old age group acts less inhibitory than serum from adult subjects. This is confirmation of the Czech investigation with *Lupinus albus L.* in 1961.

It has now also been possible to show that serum from younger subjects has a less inhibitory effect than from adult in cattle and hens, on the other hand the opposite condition has been shown in pigs.

Thus it is not a general biological phenomenon that serum from non-adult subjects acts in a less inhibitory manner than serum from adult subjects.

(3) In human individuals it has now also been shown that serum from early puerperal women has a less inhibitory effect than serum from non-pregnant and pregnant women. On the other hand it is uncertain if the serum from pregnant women acts less inhibitory than serum from non-pregnant women as determinations on individual sera showed this difference while in the present series this was not the case with determinations on pooled sera.

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Indleveret til Selskabet den 28. juli 1967.
Færdig fra trykkeriet den 15. december 1967.

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Vol. **23**, nos. 9, 12–16. – Vol. **24**, no. 2.

Printed in Denmark.
Bianco Lunos Bogtrykkeri A/S.