incubation. Figure 1, B represents the control culture without antibody. The peak to the left represents CRBC (T) while the peak to the right represents effector cells (E). The proportion of one cell type to another can be found by simply comparing the areas under the curve; in this case the E/T ratio is 12578/9833 or 1.2792. When antibody to CRBC is added to a culture some of the CRBC are lysed; as a result the proportion of CRBC to effector is decreased and the E/T ratio increases. Figure 1, A shows the change seen on a DNA histogram when anti-CRBC is added. Here the E/T ratio has increased to 21,010/3799 or 5.5304; the calculated percent kill is 77%.

Figure 2 shows the results of a time course study of ADCC by FCM using very low E/T ratios of 2/1 and less. The percent kill steadily increases over a 4-h span at all ratios resulting in a maximum kill of 77% at a 2:1 ratio and 52% at a 1:1 ratio. In addition, there is substantial killing (42%) after only 1 h at a 2/1 ratio.

When a standard chromium K cell assay was done using the same effector population we obtained 55% killing at a 50:1 E/T ratio after 4 h. Furthermore, plastic adherent cells showed enhanced cytotoxicity when measured by both FCM and chromium release[6], thus, FCM measures macrophage-monocyte killing.

This assay offers several advantages over the standard chromium release assay. First, no radiolabeling or gamma counting is needed. Second, substantial killing can be detected after only 1 h incubation compared to 4 h for the standard chromium assay. Third it is 50–100-fold more sensitive since much lower effector to target ratios than in conventional chromium assays can be used. Because of the extraordinary sensitivity of this system, this assay may be useful for the determination of antibodies against cell surface antigens, e.g., in connection with tissue typing or for detection of circulating immune complexes. Since K cell activity is mediated through the Fc receptor of the effector cell, antibody-coated cells or immune complexes can bind to effector cells and inhibit ADCC. For example, we have found that mouse and human serum containing immune complexes can significantly inhibit ADCC activity measured by FCM[7].

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Quantitative examinations of some rat proteins by means of absorbed anti-pregnancy and anti-feto sera

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Summary. This paper reports quantitative examinations of some rat serum proteins which occur only, or in rising concentrations, in pregnant rats before and after birth. A new serum protein is described which is only demonstrable in female rats. In male rats this protein is found rarely and then in very small quantities.

We examined quantitatively the development of some serum proteins of pregnant and lactating rats, as well as of young ones in their early phase of ontogenesis. This work was done in connection with research on mother-child interrelationships in the rat as a basis of quantitative and qualitative serum profile alterations.

Later on, we will report on quantitative variations of lipoproteins in the pregnant and lactating period of the rat. In this paper, we present new results concerning the quantitative behaviour of a pregnancy-associated protein (PAPP), a female specific protein (FP) and the alpha-feto protein (AFP) of the rat.

Pregnancy-associated proteins for the rat were described by Lin et al.[1,2] a few years ago. We have examined the time course of the protein which Lin et al. called PAPP-C. The position and shape of this protein peak permits a simple identification in crossed-immunoelectrophoresis (IE). Female-linked or -specific proteins are known at present for some insect species, for the hen and for the Syrian hamster[3]. Such a protein had not been demonstrated for the rat before.

Material and methods. The antisera directed against the PAPP's and against the FP were prepared according to Lin et al.[1]. Contrary to these authors, we absorbed the antisera separately with female and male serum, using normal serum, as well as lyophilized serum, for absorption purposes. Monospecific anti-AFP serum was prepared by means of absorption of an anti-feto serum with pooled serum of 8 weeks old female rats, into which turpentine was injected 48 h prior to exsanguination according to Ganrot[4] to give a high serum alpha-2-macroglobulin level. This is important for the preparation of a monospecific anti-AFP serum, because feto serum has a high amount of alpha-2-macroglobulin.

We applied the following analytical methods: rocket-IE[5], crossed-IE[6], fused-rocket-IE[7] and tandem-IE[8]. Serum of pregnant rats (21st day of pregnancy; normal rat gestation lasts about 22–23 days) was gel-filtered using Sephadex G-200. The animals, adult female and male Wistar rats of different ages, were obtained from Falcke-Barby. The fetal and young rats were obtained from the rat breeding department of the institute. The sera of the animals, pooled and single, were prepared in the usual way[1]. The immunization of the rabbits was accomplished according to the scheme of Harboe and Ingild[9].

Results and discussion. The quantitative examinations of the sera of fetal and young rats revealed a high level of AFP in 20-day-old fetuses. The protein concentration decreased with the increasing age of the young rats and was no longer demonstrable 8 weeks after birth by means of

normal rocket-IE. Possibly, the AFP of the rat is permanently demonstrable for life in very low concentrations, as it is in humans[10]. Rat AFP is present in a relatively minor concentration in the serum of pregnant females (figure 1).

The crossed-IE of pregnant rat serum (21st day of pregnancy) permitted the clear demonstration of 3 protein peaks by using anti-pregnancy serum which was absorbed with serum from normal male rats (figure 2). But we only obtained 2 precipitates (peaks 1 and 2 in figure 2) by means of anti-pregnancy serum which was absorbed with serum of non-pregnant female rats, if all other conditions were the same. In this case, peak 3 is no longer demonstrable. The 3 peaks were reliably identified by means of immunoelectrophoretical examinations (fused rocket-IE and tandem-IE) of chromatographically separated pregnant serum.

Precipitate 2 (figure 2) corresponds to the alpha-2-macroglobulin of the rat and was found in the 1st peak of the gel filtration; precipitate 3 represented the FP and was located in the 2nd peak of filtration; precipitate 1 was eluted in the 3rd filtration peak. The last protein corresponds to the PAPP-C according to Lin et al.[2] and is a pregnancy-associated protein. Figure 1 shows the quantitative development of PAPP-C in pregnant rats during the later part of pregnancy. PAPP-C is not demonstrable in serum of fetal rats with our methods.

Lin et al.[2] were successful in the demonstration of 4 PAPP's in the rat; two of them were clearly visible in the crossed-IE, in comparison to the two other weakly expressed protein peaks. We could only find 3 protein peaks under comparable conditions. 2 peaks, which were the most distinct ones, corresponded in shape and position to the PAPP-C and PAPP-A. The question whether PAPP-C is permanently present in the sera of normal animals in very low concentrations could only be answered by more sensitive methods.

The FP is clearly demonstrable for the 1st time in the serum of female rats at the age of about 2–3 weeks. The protein shows remarkable variations between different normal female rats. These individual values appear at a more even level in pregnant rats (21st day of pregnancy), FP is present in male rats only in rare cases and if so, in very low concentrations. We examined 80 sera of male rats and only two of them showed positive reactions. Pregnant females have this protein in slightly higher amounts than nonpregnant ones.

The FP of the Syrian hamster, discovered by COE[3], is hormonally regulated; therefore, it may be assumed that the rat FP also has a similar regulation. But contrary to the hamster, the rat is a sociably living animal with a pronounced order of rank[11], which is expressed by the hormone composition of each male member of a rat group[12]. Perhaps, male animals at the bottom of the social order of rank are FP-positive because they have a low testosterone level[13]. The slight increase of the FP in the sera of pregnant females and the strong variations between sera of different nonpregnant females, compared with pregnant ones, which have more even levels, may also be ascribed to hormonal influence. Alpha-2-macroglobulin is normally present in adult female and male rats in low concentrations; in pregnant females the concentration of this protein increases to about 600% at birth and then decreases to normal levels in about 4 weeks. Apart from a little temporary delay, this course also takes place for pre- and postnatal rats respectively. Young rats have low alpha-2-macroglobulin serum levels similar to the adult animals at the age of about 8 weeks.

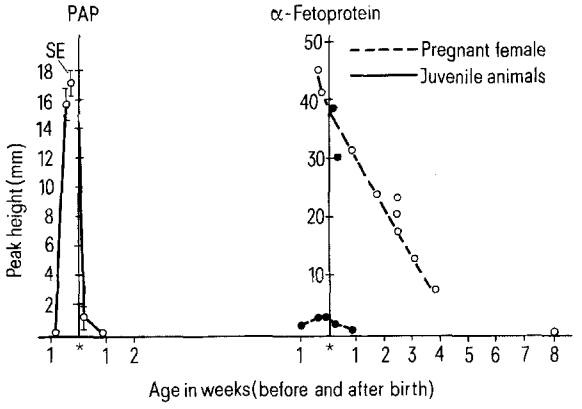

Fig. 1. The figure shows the quantitative development of rat PAPP-C (PAP) and AFP before and after birth. The open circles represent pools of serum from 10–15 single animals, the closed circles are mean values from 2–3 single animals.

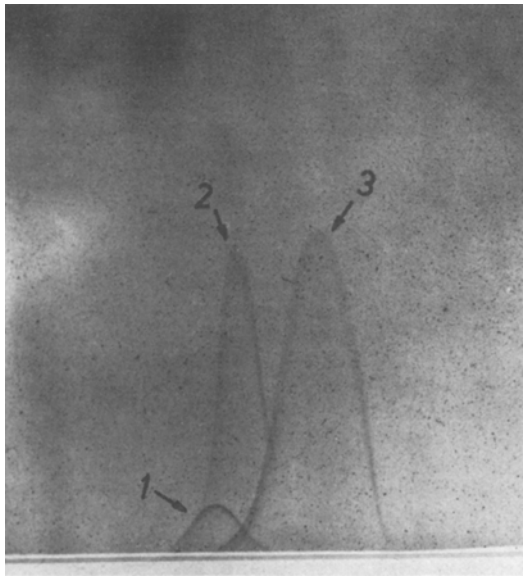

Fig. 2. Crossed-IE of serum from a pregnant rat (21st day of pregnancy). Peak 1: PAPP-C (pregnancy-associated protein). Peak 2: alpha-2-macroglobulin (acute phase protein). Peak 3: FP (female specific protein). The antiserum used was directed against serum of pregnant rat females (21st day of pregnancy) and absorbed with serum of normal male rats.

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