It is now well established that specific binding proteins for retinol and/or for retinoic acid exist in the intracellular compartment in many tissue types. The information available about these cellular retinoid-binding proteins has been reviewed recently (1). Both CRBP and CRABP have been purified to homogeneity from a number of tissues, including rat testis (2-4), bovine retina (5), and (for CRBP) rat (6, 7), dog (7), and human (8, 9) liver. Purified preparations of each of the two proteins from various sources have similar major properties. Both CRBP and CRABP have molecular weights close to 14,600 and single binding sites for one molecule of retinoid ligand. Despite considerable structural homology (10), these two binding proteins differ from each other with regard to retinoid ligand binding specificity and are immunologically distinct (1, 11, 12). They both differ in a number of major ways from the plasma transport protein, RBP (13, 14). The endogenous retinoids associated with retinoid-binding proteins from bovine retina and retinal pigment epithelium have been identified (15). It has been suggested that these intracellular proteins may play a role in the biologic expression of vitamin A activity of retinoids within cells (1, 16).

Two radioimmunoassay studies have provided information about the distribution and levels of CRBP (11, 12) and of CRABP (12) in rat tissues. The relative tissue distribution of CRBP was quite different from that of CRABP. Highest levels of CRBP were observed in the liver, kidney, and epididymis, whereas highest levels of CRABP were found in prostate, testis, and uterus.

Information is needed about the factors that regulate the levels of the cellular retinoid-binding proteins in different tissues. In this regard, an important question is whether the amount of retinoid ligand affects the level of its corresponding binding protein (1).

We now report detailed studies on the influence of retinoid nutritional status on the levels of CRBP and of CRABP found in 21 tissues of male rats. These studies employed sensitive and specific radioimmunoassays for both CRBP and for CRABP. The effects of sex were also examined by comparing the tissue distribution and levels of the retinoid-binding proteins in female and male rats.

**EXPERIMENTAL PROCEDURES AND RESULTS**

The concentrations of immunoreactive CRBP and CRABP measured in various organs and tissues from the male rats on the control diet are shown in Table II. For simplicity of presentation, portions of this paper (including "Experimental Procedures," part of "Results," Figs. 1-3, and Tables I, III, IV, and VII) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 84M-2011, cite the authors, and include a check or money order for $6.80 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

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* This work was supported by National Institutes of Health Grants AM 05368 and HL 07543. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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| Table I | The abbreviations used are: CRBP, cellular retinol-binding protein; CRABP, cellular retinoic acid-binding protein; RBP, retinol-binding protein. | Table II | The concentrations of immunoreactive CRBP and CRABP measured in various organs and tissues from the male rats on the control diet are shown in Table II. For simplicity of presentation, portions of this paper (including "Experimental Procedures," part of "Results," Figs. 1-3, and Tables I, III, IV, and VII) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 84M-2011, cite the authors, and include a check or money order for $6.80 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press. |
TABLE II

Tissue levels of CRBP and CRABP in male rats on the control diet

| Tissue                | CRBP  | CRABP |
|-----------------------|-------|-------|
|                       | pg/g, wet weight* | pg/g, wet weight* |
| Nonreproductive       |       |       |
| Liver                 | 40.0 ± 9.7 | 1.1 ± 0.2* |
| Kidney                | 26.1 ± 2.8 | 1.3 ± 0.4 |
| Lung                  | 18.9 ± 4.5 | 0.4 ± 0.1 |
| Lymph nodes           | 17.0 ± 4.6 | 3.1 ± 0.2 |
| Eye                   | 13.0 ± 3.0 | 5.2 ± 1.0 |
| Spleen                | 10.3 ± 1.4 | 0.8 ± 0.2 |
| Small intestine       | 9.9 ± 3.0 | 1.2 ± 0.3 |
| Adrenals              | 7.5 ± 1.6 | 1.0 ± 0.3 |
| Thymus                | 4.5 ± 0.5 | 1.2 ± 0.3 |
| Brain                 | 4.4 ± 1.1 | 2.5 ± 0.4 |
| Stomach               | 3.5 ± 0.6 | 1.1 ± 0.3 |
| Skin                  | 3.0 ± 0.9 | 12.6 ± 2.3 |
| Pancreas              | 2.5 ± 0.6 | 0.3 ± 0.1 |
| Fat                   | 1.2 ± 0.3 | 0.2 ± 0.1 |
| Muscle                | 1.1 ± 0.3 | 0.4 ± 0.1 |
| Reproductive          |       |       |
| Epididymis, proximal  | 67.3 ± 13.1 | 0.7 ± 0.1 |
| Testis                | 17.8 ± 4.8 | 9.5 ± 1.1 |
| Epididymis, distal    | 6.7 ± 1.6 | 5.2 ± 0.8 |
| Prostate              | 5.2 ± 1.2 | 3.8 ± 1.4 |
| Vas deferens          | 3.7 ± 1.5 | 17.4 ± 3.5 |
| Seminal vesicle       | 2.9 ± 0.7 | 30.4 ± 7.9 |

* Values represent means ± S.D. for six independently determined samples. Samples were treated as described under “Experimental Procedures.”

a Epididymis, proximal = caput and proximal corpus; epididymis, distal = distal corpus and cauda (see “Experimental Procedures”).

comparisons, the organs are grouped as either non-reproductive or reproductive. All organs and tissues examined were observed to contain measurable levels of CRBP and CRABP.

The highest concentrations of immunoreactive CRBP found in the male rats on the control diet were in the proximal portion of the epididymis, the liver, and the kidney. Values between 20 and 10 μg/g, wet weight, were found in the lung, testis, lymph nodes, eye, and spleen (and, in the female rats (see Table III in the Miniprint), in the ovary and oviducts). The mean levels in the other tissues ranged from 9.9 μg/g in small intestine to 1.1 μg/g in muscle. For immunoreactive CRABP, the highest concentrations (30.4–9.5 μg/g, wet weight) in the male rats on the control diet were found in the seminal vesicles, vas deferens, skin, and testis. Values between 6 and 2.5 μg of CRABP/g, wet weight, were observed in the eye, distal portion of the epididymis, prostate, lymph nodes, and brain. All other tissues showed values below 2 μg/g. In the female rats on the control diet (see Table IV in the Miniprint), the uterus and oviduct had mean values of 9.2 and 6.1 μg/g, respectively.

The concentrations of immunoreactive CRBP measured in the organs and tissues of male rats maintained on each of the other three experimental diets and of female rats on the control diet are listed in Table III (see Miniprint). Corresponding values for immunoreactive CRABP, measured in the same organ and tissue homogenates from the same groups of rats, are shown in Table IV (see Miniprint).

In general, the CRBP concentrations in the tissues of the rats on the retinoid acid diet and on the excess retinol diet were similar to the CRBP levels seen in the corresponding control group tissues (Table II). In contrast, the rats on the retinoid-deficient diet showed lower CRBP levels in every tissue examined, as compared to the control rats. In addition, the CRBP levels in some of the tissues of the female control rats appeared to differ from those of the male control rats.

The possible significance of the observed diet- or sex-dependent differences in tissue CRBP concentrations was examined statistically. For each tissue, the results in the five experimental groups (males on four diets and female controls) were analyzed by a one-way analysis of variance. The results of these analyses are presented in Table V. Only small intestine, stomach, brain, muscle, and the proximal portion of the epididymis did not show group-dependent effects at the 5% level. For most tissues, the CRBP levels in the rats on the retinoid-deficient diet were significantly (p < 0.01) less than the corresponding levels in the control rats. No significant differences were found between the CRBP levels in the male rats on the control, retinoic acid, and excess retinol diets. Significant differences between male and female rats on the control diet were found in liver, lung, thymus, and fat. Strikingly significant diet or sex effects (p < 0.001) were observed for the liver, lung, testis, lymph nodes, spleen, skin, and fat.

CRBP levels in the various tissues were not affected by retinoid nutritional status and did not differ among the four experimental diet groups; in addition, the levels in the female controls did not differ from those in the male controls. This was evident both by inspection of the data (Tables II and IV) and by statistical analysis (Table VI) for diet- or sex-dependent group differences. The only exception to this finding occurred with skin, where CRBP levels in the samples from rats on the retinoid-deficient diet and from female rats were both found to be significantly lower than the CRBP levels in the male control rats. No tissue differences in CRABP concentration were seen in the rats fed the retinoid acid diet as compared to control rats.

DISCUSSION

This study was designed to explore in detail the effects of differences in retinoid nutritional status and of sex on the levels of CRBP and of CRABP in various tissues and organs in the rat. The study thus aimed to investigate two of the factors (retinoid status and gender) that might be involved in the regulation of the tissue distribution and levels of these retinoid-binding proteins. Previous studies dealing with plasma RBP have shown that retinol nutritional status strikingly influences the metabolism and tissue levels of RBP (14, 20, 25). Retinol depletion and deficiency specifically inhibit RBP secretion from the liver, so that RBP levels rise within the liver, whereas RBP levels decline in plasma and in extrahepatic peripheral tissues (20, 25). Hence, the metabolism of RBP is strongly influenced by the availability of the ligand (retinol) which it normally binds and transports. Similar information has not been available previously for the cellular retinoid-binding proteins CRBP and CRABP.

The experiment reported here employed sensitive and specific radioimmunoassays for both CRBP and CRABP. These immunoassays, conducted in a similar manner, had similar characteristics with regard to sensitivity (effective detection of 1–10 ng of binding protein/assay tube), precision, and reproducibility (with acceptably low intra- and inter-assay variability). Each immunoassay employed immunofluorescence-coupled turkey antibodies specific for the respective retinoid-binding protein and showed no immunological cross-reactivity with the other cellular retinoid-binding protein, with RBP, or with liver Z-protein. The present radioimmunoassay for CRBP is approximately an order of magnitude more sensitive than the assay previously reported from this laboratory (11). Our radioimmunoassay for CRABP has not been reported previously. Somewhat more sensitive immunnoassays for CRBP and CRABP have been reported by Ong et al. (12).
Statistical analysis of diet and sex effects on CRBP level

Tissues were analyzed for diet or sex effects on CRBP level by a one-way analysis of variance. If a significant group effect was found for a tissue at the 1% significance level \((p < 0.01)\), then the mean CRBP level from each experimental group was compared to the mean level for males maintained on control diet using Dunnett’s \(t\) test (see “Experimental Procedures”).

| Tissue                  | \(p\) value for overall group effect | Retinol-deficient diet | Retinol acid diet | Excess retinol diet | Female Control diet |
|-------------------------|--------------------------------------|------------------------|-------------------|---------------------|---------------------|
| Liver                   | 0.0001 **                          |                       |                   |                     |                     |
| Kidney                  | 0.001                              |                       |                   |                     |                     |
| Lung                    | 0.0002                             | *                      |                   |                     |                     |
| Eye                     | 0.005                              | *                      |                   |                     |                     |
| Lymph nodes             | 0.0005                             | *                      |                   |                     |                     |
| Spleen                  | 0.0001                             | *                      |                   |                     |                     |
| Small intestine         | 0.31                               | NS                     | NS                | NS                  | NS                  |
| Adrenals                | 0.003                              | *                      |                   |                     |                     |
| Thymus                  | 0.003                              | *                      |                   |                     |                     |
| Brain                   | 0.10                               | NS                     | NS                | NS                  | NS                  |
| Stomach                 | 0.53                               | NS                     | NS                | NS                  | NS                  |
| Skin                    | 0.0004                             | *                      |                   |                     |                     |
| Pancreas                | 0.02                               | NS                     | NS                | NS                  | NS                  |
| Fat                     | 0.0002                             |                       |                   |                     |                     |
| Muscle                  | 0.11                               | NS                     | NS                | NS                  | NS                  |
| Epididymis, proximal    | 0.198                              | NS                     | NS                | NS                  | NS                  |
| Epididymis, distal      | 0.015                              | NS                     | NS                | NS                  | NS                  |
| Testis                  | 0.0004                             | *                      |                   |                     |                     |
| Prostate                | 0.012                              | NS                     | NS                | NS                  | NS                  |
| Vas deferens            | 0.007                              | *                      |                   |                     |                     |
| Seminal vesicle         | 0.001                              | *                      |                   |                     |                     |

** *, statistically different CRBP levels from male rats maintained on control diet; --, not statistically different from male rats maintained on control diet; NS, overall group effect not statistically significant at \(p < 0.01\).

TABLE VI

Statistical analysis of diet and sex effects on CRABP level

See legend to Table V.

| Tissue                  | \(p\) value for overall group effect |
|-------------------------|--------------------------------------|
| Liver                   | 0.72                                 |
| Kidney                  | 0.36                                 |
| Lung                    | 0.26                                 |
| Eye                     | 0.996                                |
| Lymph nodes             | 0.07                                 |
| Spleen                  | 0.89                                 |
| Small intestine         | 0.36                                 |
| Adrenals                | 0.77                                 |
| Thymus                  | 0.63                                 |
| Brain                   | 0.21                                 |
| Stomach                 | 0.33                                 |
| Skin*                   | 0.002                                |
| Pancreas                | 0.06                                 |
| Fat                     | 0.12                                 |
| Muscle                  | 0.13                                 |
| Epididymis, proximal    | 0.11                                 |
| Epididymis, distal      | 0.13                                 |
| Testis                  | 0.45                                 |
| Prostate                | 0.53                                 |
| Vas deferens            | 0.30                                 |
| Seminal vesicle         | 0.33                                 |

* Skin CRABP levels in both the retinoid-deficient and the female control groups differed significantly from those in the male control group by Dunnett’s \(t\) test.

In the previous study from this laboratory (11), it was found necessary to add both a proteolytic enzyme inhibitor and a detergent (Triton X-100) in order to effectively adapt the CRBP assay to study tissue homogenates. Triton X-100 was needed in order to render the CRBP in tissues fully immunoreactive (11, 26). In the present study, these observations were confirmed for both the CRBP and the CRABP radioimmunoassays. In the absence of Triton X-100, only approxi-
ular that the use of Triton X-100 in the homogenate samples and immunnoassays in the study reported here may have been responsible for the observed differences in results. Levels of CRBP and CRABP in several rat organs roughly similar to the levels reported here (with the notable exception of brain) have also been reported in preliminary form by Sundelin et al. (27).

Although all tissues had measurable levels of both retinoid-binding proteins, the two proteins differed greatly with regard to their tissue levels and distribution. For nonreproductive organs, the highest levels of CRBP were observed (in decreasing order) in liver, kidney, lung, lymph nodes, and brain. High levels of both proteins were observed in both the male and the female reproductive organs, with the highest levels for any rat tissue being found in the proximal portion of the epididymis for CRBP and in the seminal vesicles for CRABP. Very high levels of CRBP in the proximal part of the head of the epididymis have also been reported in other studies by Porter et al. (28) and from this laboratory (29). A significantly (p < 0.01) inverse relationship was found in the present studies between CRBP and CRABP levels in the different tissues that comprise the male reproductive tract. In both males and females, CRBP levels were highest in the gonads and the proximal portion of the reproductive tract and decreased distally, whereas the opposite was true for CRABP. This finding suggests that the two retinoid-binding proteins or their respective ligands both serve vital but very different physiologic functions within the reproductive system. The data also suggest that the two cellular retinoid-binding proteins may be localized in different types of cells in the various parts of the reproductive tract. Differential localization of CRBP and CRABP in different types of cells may also be present in nonreproductive tissues, as suggested by the significant inverse relationship in their tissue levels in the female control rats.

In this regard, more information is needed about the distribution of CRBP and CRABP within specific types of cells in the various organs and tissues. Immunohistochemical studies have shown that CRBP is localized in both parenchymal and stellate (fat-storing) cells in liver (17), in the proximal convoluted tubules of the renal cortex (17), in the Sertoli cells of the testis (29), in the ductular epithelium of the proximal part of the head of the epididymis (28, 29), in the islets of Langerhans of the pancreas (30), and in germinal centers in lymph nodes. No such information is available about CRABP. Biological perturbations that affect the distribution of cell types within tissues would be expected to affect the levels of the retinoid-binding proteins in those tissues.

Four experimental diets were used that differed greatly with regard to the kind and amount of retinoids they provided, but were otherwise identical. With regard to CRBP, the rats fed the retinoid-deficient diet (and fully retinoid-deficient by clinical and biochemical criteria) showed CRBP concentrations in tissues that were distinctly lower than those found in normal controls. The mean CRBP concentrations measured in the 21 tissues of the retinoid-deficient rats were 8-55% lower than the corresponding levels in the control rats. The data indicate that tissue CRBP levels are influenced by diet and retinoid availability, so that, in most tissue, retinoids are needed in order to maintain the normal metabolism and level of CRBP. These quantitative studies confirm and greatly extend recent immunohistochemical studies in this laboratory (17, 29) that showed decreases in specific immunostaining for CRBP in specific cells (see above) of the kidney, the testis, and the epididymis in retinoid deficiency.

The levels of CRBP in the tissues of the retinoic acid-supplemented and excess retinol-fed rats were not different from those found in the control rats. Thus, CRBP levels in tissues appear to be maintained constant in the face of considerable differences in retinoid nutritional status, other than complete retinoid deficiency. Since the retinoid acid-fed rats still had low levels of retinol in serum, it is possible that the tissues of the rats in this group were not fully depleted of retinol and that such residual retinol content played a role in preventing the decrease in tissue CRBP levels. On the other hand, the livers of the retinoic acid-fed rats were fully retinol-deficient (as indicated by their negligible retinol and elevated RBP content), yet showed normal rather than decreased CRBP levels. Moreover, the retinoic acid-fed rats had serum RBP levels that were as low as those of the deficient rats, suggesting that the rate of retinol delivery to tissues was greatly reduced. Future studies, in which rats are maintained in a retinoid-deficient state on retinoic acid for much longer periods of time, would be of interest.

Significant differences between male and female control rats were observed with 4 of 15 tissues compared (liver, lung, thymus, and fat). For each of these four tissues, the CRBP level in female rats was higher than that in male rats.

In contrast to CRBP, tissue CRABP levels showed no diet- or sex-dependent differences and were not affected by differences in retinoid nutritional status or in gender. Only in one tissue, the skin, were differences observed between experimental groups, with lower CRABP levels found in skin from retinoid-deficient and female control rats, as compared to the male rats in the other three diet groups. Thus, CRABP metabolism and levels appear to be minimally influenced by the amount or kind of retinoid ligand available or by sex.

Retinoids affect cell differentiation and proliferation and are thought to affect gene expression in target cells (31). The relationship at the cellular level between retinoid-dependent functions and the concentrations of the retinoid-binding proteins needs further exploration. The present finding that both cellular binding proteins were present in all tissues and organs examined suggests that both proteins are involved in critical biochemical functions in many types of cells throughout the body. The nature of these functions and of the regulatory processes that control the tissue levels of CRBP and CRABP remain to be determined.

Acknowledgments—We thank X. Luna, D. Orovic, R. Piantedosi, and M. Wyatt for excellent technical assistance and M. Tripptree and A. Scott for preparation of the manuscript. We are grateful to Dr. R. Ramakrishnan for the statistical analyses.

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RETIRED Status and Cellular Binding Proteins

4837

**Influence of Retinoid Nutritional Status on Cellular Retinol and Cellular Retinol Binding Proteins in Various Tissue Samples**

By

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**EXPERIMENTAL PROCEDURES**

Preparation of Polymers against CRBP and CRABP. Both CRBP and CRABP were isolated from mouse liver. The CRBP was purified by the method described by Kropp et al. (1972), representing 2,000,000-fold purification with an overall yield of 0.5%. CRABP was then purified by the method described by Kropp et al. (1972). The purified CRBP and CRABP were used as standard proteins in the assay. Turkey antisera against CRBP were first prepared by the use of a new procedure described previously (19). The IgG fraction was obtained from the turkey antisera according to the method described by Kropp et al. (1972). In addition, 100 mg of purified turkey IgG fraction was mixed with 10 mg of purified turkey IgG fraction in 5 ml of 0.5% acetic acid and stored at -20°C.

**RESULTS**

Purified turkey IgG fraction used for immunization was assayed for retinoids by means of immunodiffusion assay. The purified turkey anti-CRBP and CRABP antisera were used to purify the retinoids in mouse liver, and the purified retinoids were assayed by the method described previously (19). The IgG fraction was obtained from the turkey antisera according to the method described by Kropp et al. (1972). In addition, 100 mg of purified turkey IgG fraction was mixed with 10 mg of purified turkey IgG fraction in 5 ml of 0.5% acetic acid and stored at -20°C.

**DISCUSSION**

The results obtained from this study are consistent with previous reports in the literature. The purified turkey anti-CRBP and CRABP antisera were used to purify the retinoids in mouse liver, and the purified retinoids were assayed by the method described previously (19). The IgG fraction was obtained from the turkey antisera according to the method described by Kropp et al. (1972). In addition, 100 mg of purified turkey IgG fraction was mixed with 10 mg of purified turkey IgG fraction in 5 ml of 0.5% acetic acid and stored at -20°C.

The retinoids were assayed for their ability to bind to turkey immune sera. The purified turkey anti-CRBP and CRABP antisera were used to purify the retinoids in mouse liver, and the purified retinoids were assayed by the method described previously (19). The IgG fraction was obtained from the turkey antisera according to the method described by Kropp et al. (1972). In addition, 100 mg of purified turkey IgG fraction was mixed with 10 mg of purified turkey IgG fraction in 5 ml of 0.5% acetic acid and stored at -20°C.
Retinoid Status and Cellular Binding Proteins

The standard deviation curves for both retinoids were highly reproducible from run to run. For the CRP retinoidassay, the values for the retinoids were estimated at 2.5 and 2.5, respectively. For the CRP retinoidassay, the within-assay variability was 4.15 and the between-assay variability was 5.3.

Retinoid Nutritional Status of the Experimental Animals - The four experimental diets used in this study differed significantly with regard to the intake and amount of retinoids they provided to the rats in the different dietary groups. The retinoid (E) nutritional status of the rats was assessed by monitoring their growth rates and measuring their serum and liver levels of retinol and of ERP at the time of sacrifice. The control diet, containing 1.4 mg retinol and 1.4 mg retinol in the diet, was assigned as the standard retinoid diet. The serum and liver retinol levels of both the control and of ERP, for both rats and mice, and for the control, were within the normal range for these parameters (Table 1). (See below).

**Table 1**

| Group | Serum Retinol | Serum ERP | Liver Retinol | Liver ERP |
|-------|--------------|-----------|--------------|----------|
| Control | 7.9 ± 4.1 | 1.5 | 10.6 ± 4.4 | 1.5 |
| Retinol | 10.8 ± 4.1 | 1.5 | 12.7 ± 4.5 | 1.5 |
| ERP | 12.5 ± 4.1 | 1.5 | 14.6 ± 4.5 | 1.5 |
| Control | 28.8 ± 15.3 | 1.5 | 30.6 ± 15.3 | 1.5 |
| Retinol | 19.3 ± 15.3 | 1.5 | 20.6 ± 15.3 | 1.5 |
| ERP | 31.0 ± 15.3 | 1.5 | 32.3 ± 15.3 | 1.5 |

* Values represent mean ± S.D. for all six experimental animals from each dietary group. Retinol and ERP in mg were described as “Experiment Protocol.” The diet for each group is listed below.

**Table 2**

| Group | Serum Retinol | Serum ERP | Liver Retinol | Liver ERP |
|-------|--------------|-----------|--------------|----------|
| Control | 7.9 ± 4.1 | 1.5 | 10.6 ± 4.4 | 1.5 |
| Retinol | 10.8 ± 4.1 | 1.5 | 12.7 ± 4.5 | 1.5 |
| ERP | 12.5 ± 4.1 | 1.5 | 14.6 ± 4.5 | 1.5 |
| Control | 28.8 ± 15.3 | 1.5 | 30.6 ± 15.3 | 1.5 |
| Retinol | 19.3 ± 15.3 | 1.5 | 20.6 ± 15.3 | 1.5 |
| ERP | 31.0 ± 15.3 | 1.5 | 32.3 ± 15.3 | 1.5 |

* Values represent mean ± S.D. for all six experimental animals from each dietary group. Retinol and ERP in mg were described as “Experiment Protocol.” The diet for each group is listed below.

**Table 3**

| Group | Serum Retinol | Serum ERP | Liver Retinol | Liver ERP |
|-------|--------------|-----------|--------------|----------|
| Control | 7.9 ± 4.1 | 1.5 | 10.6 ± 4.4 | 1.5 |
| Retinol | 10.8 ± 4.1 | 1.5 | 12.7 ± 4.5 | 1.5 |
| ERP | 12.5 ± 4.1 | 1.5 | 14.6 ± 4.5 | 1.5 |
| Control | 28.8 ± 15.3 | 1.5 | 30.6 ± 15.3 | 1.5 |
| Retinol | 19.3 ± 15.3 | 1.5 | 20.6 ± 15.3 | 1.5 |
| ERP | 31.0 ± 15.3 | 1.5 | 32.3 ± 15.3 | 1.5 |

* Values represent mean ± S.D. for all six experimental animals from each dietary group. Retinol and ERP in mg were described as “Experiment Protocol.” The diet for each group is listed below.

**Figure 1**

Figure 1: Mean growth curves of each of the five experimental groups of rats, for the control and of retinol and of ERP, for the control, were within the normal range for these parameters (Table 1). (See below).

**Figure 2**

Figure 2: Mean growth curves of each of the five experimental groups of rats, for the control and of retinol and of ERP, for the control, were within the normal range for these parameters (Table 1). (See below).

**Figure 3**

Figure 3: Mean growth curves of each of the five experimental groups of rats, for the control and of retinol and of ERP, for the control, were within the normal range for these parameters (Table 1). (See below).