EXTRACTION AND ISOLATION OF GAMMA GLOBULIN FROM 
THE BOVINE THYMUS GLAND

SYED KAMAL AND C. W. TURNER
Department of Dairy Husbandry
University of Missouri, Columbia

The high mortality rate in young animals is a serious economic problem in the livestock industry in general and the dairy industry in particular. In addition to the use of improved methods of management, feeding and breeding, it is important to consider the inherent deficiency of the newborn animal as regards the immune globulin content of its blood. Nature overcomes this deficiency by providing the newborn animal with antibody-rich, immune lactoglobulin in its dam’s colostrum. Furthermore, the immunity imparted through colostrum feeding is of a transitory nature and the young animal is more susceptible to diseases until such time as its own gamma globulin content of the blood attains a normal level. The possibility of overcoming this inherent deficiency by supplying the newborn animal with immune globulin in addition to colostrum feeding, either from the blood or from other tissues, seems feasible.

It was proved over half a century ago that specific antibodies to nursing mice were transmitted in the colostrum (6). Also, it has been demonstrated that placental transmission plays no important role in ruminants and that the transmission of immunity is mainly through colostrum feeding in newborn animals (8, 16, 18).

The blood serum of newborn calves is deficient in globulin and such animals, if not allowed to suckle, are unusually susceptible to colon bacillus septecemia (23). Furthermore, it has been shown by means of electrophoretic studies that the blood of a newborn calf lacks immune globulin and that an immediate increase in the gamma globulin content of the blood of newborn calves occurs following the ingestion of colostrum during the first 24 hr. (9, 12). The fact that in young animals the serum protein values are normally below adult values has a possible bearing upon the increased susceptibility of young calves to many infectious agents (4).

Numerous investigators have demonstrated that the lymph glands are sites of antibody formation (5, 13, 19). Many investigators also, have shown the similarity and inseparable nature of antibodies from immune globulin with which they are invariably associated (2, 4, 5, 22, 24).

Recent endocrine work indicated that lymph glands such as the thymus of small animals contained gamma globulin (25). The gamma globulin was thought to be released and regulated by the adrenal cortical hormones. It was thought that these tissues might serve as a rich source of gamma or immune globulin. Therefore, attempts were made to extract gamma globulin from the bovine thymus.

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EXPERIMENTAL MATERIAL AND METHODS

Bovine thymus glands were obtained from the packing house immediately after slaughtering the animals. The glands were carefully dissected, fascia and fat being removed. These then were weighed into 50 g. lots, wrapped in butter paper and kept at -15 ° C.

Acetone-dried and ether-defatted thymus tissue used in these experiments was prepared mainly according to the method described by Bergman and Turner (1) for the preparation of dehydrated pituitary powder except that the procedure was carried out at -5 ° C. to avoid denaturation of the thymus tissue proteins.

Calf thymus dessicated at 40° C. used in these experiments was obtained from the Viobin Corporation, Monticello, Ill.

The procedure followed for the extraction of salt soluble proteins from the thymus glands was according to that described by Luck (17) for the extraction of proteins from liver. One molar NaCl solution was used to dissolve thymocytes and pH was adjusted to 5.0 with 0.05 M acetic acid to precipitate nucleoproteins, as recommended by Mirsky and Hans (20). Thymocytes also were extracted with a 20 per cent solution of NaCl according to the method described by White and Dougherty (35).

Mincing of the thymus tissue and stirring for extraction of the minced tissue was carried out in the cold, whereas centrifugation for the recovery of supernatant from the saline tissue extract was carried out at 3500 R.P.M. for one hr. at room temperature in the absence of a refrigerated centrifuge.

The general scheme followed for the separation of gamma globulin from the saline thymus extracts was according to the ethanol precipitation procedure described by Hess and Deutsch (11) for the serum of normal cows. The precipitation of gamma globulin from the saline thymus extracts also was attempted with ammonium sulfate, according to the procedure described by Cohn and coworkers (3) for the normal serum of the horse.

RESULTS

Only 60 mg. of acetone-dried gamma globulin was obtained from a 20 per cent saline extract of 50 g. of frozen bovine thymus by precipitating it at 34 per cent saturation with ammonium sulfate at pH 6.0. The ethanol precipitation procedure of Hess and Deutsch was ineffective in recovering gamma globulin.

DISCUSSION

It was not possible to extract and precipitate an appreciable quantity of gamma globulin from bovine thymus with either ethanol or salting-out procedures usually employed for blood. The negative results in these experiments may be explained as follows: First, there may be species differences. The hypothesis advanced by White and Dougherty (25) that the lymphoid tissues in small animals (rabbits and mice) are store houses of gamma globulin may not apply to large animals. Second, other investigators have failed to duplicate the results of White and Dougherty (25) in small animals. It has been shown that the level of serum albumin in the rat is under the control of the adrenal cortex (14). Also,
it has not been found possible to obtain evidence that adrenotrophic hormone causes a significant elevation in the concentration of the globulin fractions of the plasma or lymph of rats treated with adrenotrophic hormone (15). Furthermore, adrenal cortical activity in the rat is not essential for the fabrication or release of antibodies and gamma globulin (11). It also has been shown that adrenalectomized rabbits with hypertrophy of the lymphoid tissues produce antibodies far in excess of that produced by intact animals (21).

A very recent comparative electrophoretic and ultracentrifuge study has been made of the saline extracts of lymphocytes from popliteal (regional) lymph nodes of the hind feet of rabbits infected with killed dysentery organisms (10). The components with higher electrophoretic mobilities were increased after the injection of antigen, whereas the gamma globulin was not increased significantly.

The presence of a component of the same electrophoretic mobility as the gamma globulin of the blood in the lymphoid cells, as reported by White and Dougherty (25), also has been demonstrated by other investigators (10, 13). Therefore, there can be no question as to the presence of gamma globulin in the lymphoid cells, but these studies indicate that the amounts present in bovine thymi are not present in sufficient amounts to be extracted and precipitated by the procedures employed.

SUMMARY

1. Fresh bovine thymus glands, acetone-dried and ether-defatted thymus tissue, and desiccated calf thymus tissue were extracted with 1 M saline solution. No gamma globulin could be precipitated from these extracts either by adjusting the pH to 7.7 and ethanol concentration to 18 per cent by volume at -10°C, or by ammonium sulphate at 34 per cent saturation at pH 6.

2. Fresh bovine thymus glands were washed three times with physiological saline and lysed with one volume of distilled water and then extracted with one volume of 20 per cent saline. The saline extract yielded 60 mg. of acetone-dried gamma globulin on 34 per cent saturation with ammonium sulphate at pH 6.

3. It is concluded that the bovine thymus, though containing small amounts of gamma globulin, cannot be used as a rich source of immune globulin.

REFERENCES

GAMMA GLOBULIN FROM THYMUS GLAND


