GENERAL CONSIDERATION

More than four decades ago it became known that there could be basic differences in the patterns of metabolism of the liver of male and female rats (Greisheimer, 1931). Since then several workers (Okey et al., 1934; Barnes et al., 1941; Best et al., 1951; Aftergood et al., 1957; Fillios, 1957) have reported their observations on several sex-dependent differences in various metabolites and metabolic processes of mammalian liver. In the more recent years it was reported that lack or excess of hormonal substances leads to significant alterations in the normal hepatic metabolic patterns (Doeg, 1968; Konopkova and Nedvidek, 1972; Khandwekar et al., 1973). It is needless to make a special mention of already existing voluminous literature on the effects of castration and subsequent replacement therapy on varied tissues and organs. A cursory survey of this literature brings forth that almost all these studies report on effects observable after few to several weeks of castration and on influences of considerably long regimens of hormonal replacements. Another point of significance worth noting is that very

recent research work clearly hints at very rapid effects of hormones on certain of the fundamental biochemical processes, even those involving nucleic acids (Liao et al., 1965; Fuji and Villee, 1968). A tangential observation concerning induction of polyploidy in hepatic nuclei due to castration (Konopkova and Nedvidek, 1972) becomes meaningful in this context. Considering all these facts it was difficult to ignore the desirability of knowledge regarding immediate effects of castration and replacement therapy on the metabolic adjustments in the mammalian liver. It was with this view that the present investigation was undertaken. As is common in several experimental studies, here also arbitrary intervals of 1, 2, and 5 days were chosen in looking for early effects of castration. In regard of replacement studies initially it was thought desirable to try out lowest possible dose-range in order to find out minimal requirement for restoration. Such an influence of hormone replacement was assessed after 24 hrs. of administration.

As a part of this investigation (Chapters-1 to 7) several important metabolites viz., total lipids, cholesterol, phospholipids, free fatty acids (FFA),

glycogen, proteins, nucleic acids and ascorbic acid (AA) and certain important enzyme activities viz., phosphorylase, glucose-6-phosphate dehydrogenase (G-6-PDH), succinate dehydrogenase (SDH), adenosine triphosphatase (ATPase), acid- and alkaline phosphatase were studied under the experimental conditions outlined in the immediately preceding paragraph. Certain parameters viz., rate of fatty acid oxidation in liver, plasma lipids, plasma total cholesterol, plasma phospholipids, plasma FFA and FFA of omental fat depot showed maximum significant alterations after 48 hrs. of castration, hence, the influence of replacement therapy was studied by injecting the hormone to 48 hr$. castrates. The effect of hormone replacement was observed at 1, 2 and 4 hrs. after administration to understand immediate effects on sensitive metabolic patterns of rat liver.

Before going further into details of the observations and interpretations it is felt necessary to mention here another important assumed consideration that was a part of background of this study. It was suspected that there could exist some sort of regional differences between different lobes of the rat liver. All the four lobes of the liver of rat, therefore, were studied separately in

the initial stages of inquiry. Soon the fact of regional difference was borne out, though in a peculiarly restricted sense. All the lobes of the liver were more or less similar in several respects except the Spigelian lobe. This lobe stood out well in almost all aspects, except normal content of glycogen. The Spigelian lobe varied differently from other lobes under all the experimental treatments ~~of~~ followed here. This obviously indicated that the Spigelian lobe exhibits a sensitivity to fluctuations in hormonal levels in a manner distinct from other liver lobes. In the light of these findings it was decided to carry out further studies separately for the Spigelian lobe and the median lobe, the latter being a representative of the rest of the liver lobes.

Interest in estimating the contents of important metabolites mentioned was to reveal possible loci for further study. Coupled with these observations those on alterations of certain of the concerned enzymes would certainly aid better understanding of the problem.

A perceptible hyperglycemia was observed to occur at 48 hrs. through 120 hrs. (Chapter-9). A simultaneous and corresponding gradual fall in hepatic glycogen content

was evident (Chapter-3). Liver phosphorylase activity did exhibit appropriately higher levels. G-6-PDH was observed to be significantly suppressed due to castration, indicating reduced rates of direct oxidation of glucose by the liver. SDH activity in liver was seen to remain the least altered parameter (an enigmatic but significant lowering was noted only in case of the Spigelian lobe by 24 hrs. of castration). This indicated no influence on the rate of turnover of Tricarboxylic acid (TCA) cycle and hence involved oxidative metabolism of glucose by the liver itself. It is, therefore, apparent that existance of a subtle check on the rate of hepatic glycogenolysis and subsequent release of glucose into the blood by the circulating androgenic titres could be a possibility. From the results obtained within first four hours after injecting 0.1 mg of testosterone propionate (TP) to 48 hrs. castrates; it could be seen that a drastic hypoglycemia developed within first sixty minutes but was gradually reduced to a safer limit within four hours. Under these conditions, however, the hepatic glycogen content did neither exhibit significant variation nor an understandable pattern of fluctuation, that could be

elucidated on the basis of present data. As against this, distinct reduction in liver phosphorylase activity was seen to be brought about with the single injection of TP within first hour and retained that low through the 4 hr. period of observation. Additionally, SDH activity was markedly reduced to less than 50% of normal levels and similarly maintained at that low level through four hours. Among the two lobes the Spigelian lobe showed a distinctly greater sensitivity under these experimental conditions. Taken together, it means the replacement induces an altered reparative process, involving reduction in glycogenolysis but an increase in oxidative utilization by hepatic tissue. The influence of replacement appeared to be rather of a striking nature, however, it is not possible to offer a plausible explanation of this occurrence on the basis of limited data at hand. Nevertheless, it is difficult to restrain the expression of demanding nature that the finding proclaims.

Castration was observed to induce after 24 hrs., a perceptible increase in the total lipid (glycerides) content but a distinct decrease in the levels of cholesterol as well as phospholipids (Chapters-1 & 2).

Responses exhibited in FFA levels by the median and Spigelian lobes are contrasting, the former registering an increase and the latter a decrease (Chapter-2). FFA level in the blood plasma revealed marginal lowering. Omental lipid depot was found to show a marked rise in the level of FFA (Chapter-8). Total glyceride content showed a gradually increasing tendency upto 120 hrs. Total cholesterol and phospholipid concentrations were found to be raised significantly after 48 hrs. FFA level in both of the liver lobes and blood plasma went up but, contrastingly enough, the omental FFA level was very low. These findings indicated an initial loss of cholesterol and phospholipids from the liver. Plasma also depicted a concomittant fall in the levels of these two constituents (Chapter-9). Simultaneously, a more than 50% reduction in the hepatic capacity for fatty acid oxidation was revealed in 24 hrs. castrates with further reduction by 48 hrs. (Chapter-8). These findings, therefore, indicated an accelerated release of cholesterol and phospholipids from the liver and an increased mobilization of FFA in omental fat pad. In these circumstances it may be suggested that the liver takes very little of plasma FFA showing marginal increase in esterification thereof. By

48 hrs. after castration the whole pattern was altered. Net triglyceride gain by liver was more alongwith greater accumulation of phospholipid and cholesterol. Lowering of FFA from omental store, increase of the same in blood plasma, and yet lower fatty acids oxidizing capacity of liver, logically point to shifting of FFA from depot to liver with concomittant incorporation into triglycerides and phospholipids. At 120 hrs. of castration glyceride content improved whereas cholesterol and phospholipids showed a noticeable decrease. At this stage, omental mobilization of FFA could easily be seen. A sudden depletion of plasma FFA indicated faster rate of removal at some other sites, one of which could be liver itself, as it showed increased glyceride content with the least changes in phospholipid and cholesterol levels and also improvement of its capacity for fatty acid oxidation. At this stage plasma registered falling levels of all the three parameters. Castration, therefore, led to fat accumulation in the liver through a period of five days after castration. With the three different dosages of TP that were administered 24 hrs. post-operatively no very significant variation was observable yet, 0.1 mg dose was found to be sufficient to bring about restoration.

Maximum fluctuations in almost all the parameters were observable 48 hrs. post-operatively. Here the very same dose was found to be insufficient, as was the case in all preceding parameters. However, when the influence of 0.1 mg TP was assessed at 1, 2 and 4 hrs. after administration to 48 hr. castrates the following points were found noteworthy:-

During the first 60 minutes fatty acid oxidizing capacity of both of the liver lobes improved but that of the Spigelian lobe was almost fully restored to normal level. Lipid level of blood plasma was slightly raised but the levels of plasma cholesterol and phospholipids were very significantly lowered.

For the first two hours FFA levels of blood plasma and omental depot remained low. During second hour fatty acid oxidizing capacities of both the liver lobes registered higher levels. Blood plasma levels for total lipids, cholesterol and phospholipids improved considerably.

By 4 hrs. of injection the fatty acid oxidizing capacities of both the lobes were held at higher levels. A very marked rise was noticed in the FFA levels of omental depot and blood plasma and those for phospholipids

and cholesterol shifted towards normal. Interestingly enough, within 4 hrs. after administration of TP, no appreciable changes could be seen to occur in the FFA levels of liver lobes themselves. These were raised higher than normal by castration and the same could not be brought down to normality as long as four hours after injection of hormone. It appears, therefore, that probably the greater bulk of FFA of plasma might get diverted into glyconeogenetic pathway in the liver and/or towards uptake by peripheral tissues. SDH activity was reduced to less than 50% of the normal value in the liver at this hour whereas glycogen level was higher than normal and the phosphorylase activity too, was found to be drastically low (Chapter-11). It has been shown by Weber (1967) that higher concentration of fatty acids suppresses glycolysis and subsequent oxidation/phosphorylation but enhances glyconeogenetic system leading to a rise in glycogen levels of rat liver. This, therefore, might explain the present findings to a certain extent. It is not, however, possible to explain of what significance the quick restoration of fatty acid oxidizing capacity was under these circumstances. The only possibility could be that restoration of overall oxidative metabolic capacity

of hepatic tissue might take more than 4 hrs., which was unfortunately not observed presently.

Normal rat liver is known to synthesize and retain ascorbic acid (AA) (Hassan and Lehninger, 1956). Participation by AA in the general oxidation-reduction processes of various tissues has been suggested by Meiklejohn (1953) and later elaborated by several workers. Involvement of AA in steroidogenesis in varied ways has been discussed by Szent Gyorgyii (1957) and Bacq and Alexander (1961). It is known that normal maintenance of carbohydrate, lipid and iron metabolism demands the presence of certain physiological levels of AA in the tissues. In other words fluctuations in AA content disturb these metabolic processes. Additionally, there is ample evidence for the existence of an intricately dynamic interrelationship between AA synthesizing capacity of the liver and the circulating androgen levels (Dieter, 1969; Majmudar and Chatterjee, 1974; Chinoy and Parmar, 1975; Chinoy et al., 1975a,b). Taking these facts into account it is not difficult to expect significant changes in hepatic AA content that may be brought about by castration and hormone administration. Several reports on these

aspects are available (Stubbs and McKernan, 1967; Stubbs et al., 1967 and Khandwekar et al., 1972).

Present investigation has brought out heretofore unsuspected alteration in AA metabolism. It was observed that sham operation led to significant reduction in AA content of both the liver lobes. As against this the AA levels in these two lobes registered a much greater rise after 24 hrs. of castration (Chapter-5). This rise was retained upto 48 hrs. but later by about 120 hrs. it was noted to be reduced significantly close to normal levels in case of both the liver lobes. Spigelian lobe, however, exhibited higher AA content under all experimental conditions and also a comparatively high sensitivity to experimental changes than the median lobe. Replacement with hormone was observed to counteract the initial effect of castration as expected. A noteworthy point in this connection was that even after as late as 120 hrs. of castration the hepatic tissue responded well to 0.1 mg dose of TP. This probably points to an independent behaviour of AA component of biochemical environment in the hepatic tissue, though it is influenced by fluctuations in the levels of circulating hormone. Such an exclusive

nature of AA may presumably help the tissue tide-over initial lowering of general oxidative metabolism through its participation in oxidation-reduction processes. Nevertheless, this substitution is obviously a temporary adjustment, as later on a return to normality was evident. From the observations recorded here, however, there was no discernible evidence of influence of AA on hepatic cholesterol metabolism during these early modulations.

Considering the multiferious alterations that were noted to occur under the experimental conditions employed here it was logical to expect appropriate fluctuations in the overall energy levels in the hepatic tissue. Contrary to this assumption no significant variation could be observed in the levels of Mg^{++} dependent adenosinetriphosphatase (ATPase) activity (Chapter-6), which is well known to be intimately associated with general energetics of the cells. The influence of castration was to slightly lower this enzyme activity and hormone administration could bring about restorative changes. Mere absence of appreciable changes in the levels of ATPase activity does not warrant non-attribution of any role to it but the very lack of measurable changes equally well does not permit

any proffering of particular explanation of the circumstance. It would, therefore, be premature to pass any comment on the functional variation of Mg^{++} depedent ATPase activity in the present context.

Though it is quite common-place that castration and gonadal dysfunction lead to the development of certain characters not found in the normal individuals, yet the exact nature of these is not understood to any appreciable extent. The metabolic patterns of normal intact individuals logically must be of certain nature which are likely to undergo subtle alterations when the gonads are removed in adult condition. Expression of such changes may find manifestation in diverse ways. It is, therefore, quite possible that gonadal hormones, that were normally playing a role in the expression of normal genetic patterns, on being removed, could no longer influence such a process. This may lead to changes in basic biochemical repertoire due to alterations in the activity of genes at different loci. It is desirable to focus attention on some of the basic parameters that would permit more meaningful statements than hitherto made. As was referred to in chapter(4); evidence was available that

castration induces polyploidy in the hepatic cell nuclei in rats. On the basis of these reports and above mentioned reasoning it was deemed necessary to study changes in the concentrations of deoxyribonucleic acid (DNA) as well as ribonucleic acid (RNA) under the experimental conditions adopted in the present inquiry. Simultaneous changes in the total protein content would probably add to the information, hence, total protein contents were also measured.

It was observed that castration led to a significant rise in the concentrations of DNA, RNA as well as total protein. By 120 hrs. of castration tendency towards return to normality was just perceptible in the nucleic acid levels. Administration of hormone to castrates induced only a temporary restoration for less than 24 hrs. of replacements. This suggested that, perhaps, for a desirable normalization of nucleic acids and proteins a certain minimum but sustained titre of male sex hormone is essential. If that be so, then the assumption that subtle alterations are implicated in castrated animals becomes plausible. In these circumstances return to original normality can not be sustained. Elevations in the levels of RNA and total proteins during first few days of castration may possibly fore-shadow biosynthesis of certain altered categories of

enzymic proteins. Presently obtained data clearly do not permit more than mere conjecture, however, analysis of chemical properties of surplus proteins would certainly help to clarify this situation.

It may be mentioned in this particular context, with certain emphasis, that though the Spigelian lobe exhibited a different response than that of the median lobe, significance of this difference was beyond clear understanding.

From the observations on non-specific phosphomonoesterase activity, at both the acid and alkaline ranges, it was seen that castration induced decrease of the former and increase of the latter type of enzyme activity (Chapter-7). Under the influence of TP administration to 24 hrs. castrates the acid phosphatase activity could be restored to normal levels but that of the alkaline phosphatase was not influenced noticeably by any of the three different doses. It naturally means that the changes induced in the case of alkaline phosphatase activity are less reversible in nature. On the other hand, when 0.1 mg. of TP was administered to 120 hrs. castrates it was alkaline phosphatase activity that attained levels close to normality whereas that of acid phosphatase was observed to be adversely affected. It appears, therefore,

that during first two days of castration certain changes occur as a result of lack of sex hormone but later by about five days an altered pattern of sensitivities and metabolic processes start manifesting themselves.

Of recent a lot of interest seems to centre around multifarious biochemical role of cyclic adenosine-3', 5'-monophosphate (cAMP) in the modulation of several cellular functions. It has been ascribed the position of a secondary messenger in biochemical integration. Several hormonal actions are known to involve cAMP. It was thought worthwhile to look for probable involvement of cAMP in the rat hepatic tissue in relation to male sex hormone. However, with the facilities available, it was technically not possible to assay directly the concentration of cAMP. Since it is well known that there exists a phosphodiesterase activity highly specific for cAMP, an attempt was made to measure this enzyme activity. This phosphodiesterase activity reduces cAMP to 5'AMP very effectively within extremely short time. So a measure of the activity of this enzyme could easily reflect cellular levels of cAMP.

It was observed that sham-operation had no effect on the enzyme activity. Castration decidedly lowered

the enzyme activity in both the liver lobes upto 120 hrs.; the median lobe, however, registered a tendency to return towards normality by then (Chapter-10). Low phosphodiesterase activity would allow cAMP to accumulate, if formed under the circumstances. This would allow stimulation of protein kinase leading to increasing levels of cellular protein content. This may be considered as a passive response since it was clear that injection of TP induced further lowering of phosphodiesterase activity. This would enhance the action of cAMP on cellular systems due to a two-pronged effect, firstly by induction of cAMP formation, as is known in case of action of androgens (Singhal and Valadares, 1968; Singhal et al., 1968; Santti and Ville, 1971; Singhal et al., 1971; Mangan et al., 1973) , and secondly by reducing the rate of its removal through suppression of phosphodiesterase activity. In the present context it could be recalled that under similar influence of TP, hepatic phosphorylase activity too, was lowered very markedly, possibly indicating modulation due to extended cAMP action. Thus, it seems that physiologically maintained circulating levels of androgenic compounds under normal conditions apparently influence intracellular cAMP concentrations not only by stimulating membrane bound cAMP

synthetase activity but possibly also through regulation of phosphodiesterase activity. The latter action appears to be similar to that of insulin, through cAMP, on phosphorylase system.

The present study highlights the necessity of a more thorough investigation of early effects of castration and replacement therapy. As was shown certain changes, viz., concentration of nucleic acids, proteins, AA, levels of phosphorylase, phosphodiesterase activities and hepatic capacity for fatty acid oxidation, virtually demand more attention since these parameters did depict in a period of 48 hrs. such variations as were contrary to those notable after longer post-castration intervals. The main suggestion is, though the castrated animals continue to survive, that there are certain alterations at the biochemical level and that this new pattern appears to get established after about five days post-operatively.

Before concluding this epilogue it should be confessed that during the course of arbitrary selection of various time intervals for assessment of the influence of hormone replacement certain lacuane in information-gathering have

crept up inadvertantly. The Author wishes to confess his inability in filling up those lacunae within the permitted time limits that were available, but expressly hopes to do this needful at the first available opportunity. Precisely for this reason speculative arguments have been kept to a minimum, though at places wherever felt necessary such indulgence was resorted to with caution merely for the sake of emphasizing a particular point. It may be mentioned at this end that this investigation has raised more questions than answering even a few, for which the author feels entitled to hold to a desire to get proper time and suitable facilities in future to clear this aftermath as far as possible.