CHAPTER VII

DISCUSSION
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Cholesterol contents of the liver of rat under different conditions.

(Table Nos. 1 (a), (b), (c) and (d) : Graph No. 1)

The cholesterol contents of the liver of rat vary with different conditions. When the rats are starved for 72 hours, the cholesterol is increased by 1.21%. Infection of Cysticercus fasciolaris also increases cholesterol of liver by 11.51%. When $\Delta^9$-Tetrahydrocannabinol ($\Delta^9$-THC) is injected into the peritoneal cavity, the cholesterol contents of the liver are highly enhanced as much as 72.54%. Since the injections of $\Delta^9$-THC are comparable to smoking of "ganja" (Cannabis sativa tops), the smoke of this narcotic will thus lead to an increase of cholesterol in the liver not only in the rats but most probably also in the human beings.
Cholesterol contents of the spleen of rat under different conditions.

(Table Nos. 2 (a), (b), (c) and (d) : Graph No. 2)

The cholesterol contents of the spleen are increased only in starving conditions by as much as 26.24%. In other conditions they are reduced. For example, in the rats infected with *Cysticercus fasciolaris*, the reduction is 26.25% while in the rats injected with $\Delta^9$-Tetrahydrocannabinol, the reduction is of the order of 36.23%.
Cholesterol contents of the testis of rat under different conditions.

(Table Nos. 3 (a), (b), (c) and (d) : Graph No. 3)

The cholesterol contents of the testis of rat increase under all the different conditions examined in this study. The increase is as follows:

- Starvation: 27.07%
- Infection of Cysticercus fasciolaris: 25.10%
- Injections of $\Delta^9$-Tetrahydrocannabinol ($\Delta^9$-THC): 46.78%
- Injections of Cannabidiol (CBD): 195.95%

The effect of Cannabidiol (CBD) is remarkable since the increase in the cholesterol contents of the testis is very high. Since the smoke of Cannabis sativa contains both $\Delta^9$-THC and CBD, the synergistic effect may perhaps be still higher.
Cholesterol contents of the adrenal of rat under different conditions.

(Table Nos. 4 (a), (b), (c) and (d) : Graph No. 4)

The cholesterol contents of the adrenal are reduced only in the infection of Cysticercus fasciolaris by 28.57%. In other conditions there is increase in varying proportions as follows:

- Starvation: 18.51%
- Injection of $\Delta^9$-Tetrahydrocannabinol: 171.42% ($\Delta^9$-THC)
- Injections of Cannabidiol (CBD): 64.28%
Effect of amino acids, dopamine and stearic acid on cholesterol production in the liver of rat.

(Table Nos. 5 (a), (b) ; Graph No. 5)

In normal rats, alanine, \( \alpha \)-aminobutyric acid, arginine, aspartic acid, cysteine, cystine and DOPA increase the quantity of cholesterol in in vitro studies with liver tissue. \( \alpha \)-aminobutyric acid and arginine increase the quantity by 57\% and alanine does the same by only 14\%. Glutamic acid, glycine, histidine, hydroxyproline, leucine, isoleucine, nor-leucine, lysine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine reduce the quantity of cholesterol in different proportions. Histidine, lysine, tryptophan and valine reduce the maximum, that is, 43\% while nor-leucine reduces it to the minimum, that is, by 8\% only. Other amino acids stand in between these two percentages. Methionine and ornithine do not have any effect on the quantity of cholesterol. It remains the same as the control. Dopamine and stearic acid reduce the quantity only by 15\% and 8\% respectively.
Effect of amino acids, dopamine and stearic acid on cholesterol production in the spleen of rat.

(Table Nos. 6(a), (b); Graph No. 6)

In normal rats, phenylalanine and valine increase the quantity of cholesterol in in vitro studies with the spleen tissue. Valine increases the quantity by 33% and phenylalanine does the same by only 11%. Alanine, α-aminobutyric acid, arginine, aspartic acid, cysteine, cystine, glutamic acid, glycine, histidine, leucine, isoleucine, nor-leucine, lysine, methionine, ornithine, proline, serine, threonine, tryptophan and tyrosine reduce the quantity of cholesterol in different proportions. Methionine and ornithine reduce the maximum, that is 33% while α-aminobutyric acid, isoleucine, tryptophan and tyrosine reduce it to the minimum, that is 6% only. Other amino acids stand in between these two percentages.

DOPA does not have any effect on the quantity of cholesterol. It remains the same as the control. Dopamine and stearic acid increase the quantity of cholesterol by 22% and 11% respectively.
Effect of amino acids, dopamine and stearic acid on cholesterol production in the testicular tissue of rat.

(Table Nos. 7 (a), (b) : Graph No. 7)

In normal rats, alanine, \(\alpha\)-aminobutyric acid, ornithine, serine and tyrosine increase the quantity of cholesterol in in vitro studies with testicular tissue. Alanine, \(\alpha\)-aminobutyric acid, serine and tyrosine increase the quantity by 66% and ornithine does the same by 33% only. Arginine, cysteine, Cystine, DOPA, glutamic acid, glycine, histidine, leucine, lysine, phenylalanine, proline, threonine, tryptophan and valine reduce the quantity of cholesterol in different proportions. DOPA, glutamic acid, glycine, leucine, lysine, phenylalanine and threonine reduce the maximum, i.e., 34% while proline reduces it to the minimum i.e., 9% only. Other amino acids stand in between these two percentages. Aspartic acid, isoleucine, nor-leucine and methionine do not have any effect on the quantity of cholesterol. It remains the same as the control. Dopamine also does not have any effect on the quantity of cholesterol while stearic acid reduces it by 17%.
Effect of amino acids, dopamine and stearic acid on cholesterol production in the adrenal tissue of rat.

(Table Nos. 8 (a), (b); Graph No. 8)

In normal rats, cysteine, cystine, DOPA, glutamic acid, glycine, leucine, isoleucine, nor-leucine, ornithine, phenylalanine, serine and valine increase the quantity of cholesterol in in vitro studies with the adrenal tissue. Phenylalanine increases the quantity by 400% and cysteine, cystine, DOPA, glutamic acid, glycine, leucine, nor-leucine, ornithine, serine and valine do the same by 100% only. Isoleucine increases the quantity by 300%. α-Aminobutyric acid reduces the quantity by 50%. Alanine, arginine, aspartic acid, histidine, lysine, methionine, proline, threonine, tryptophan and tyrosine do not have any effect on the quantity of cholesterol. It remains the same as the control. Dopamine increases the quantity by 50% while stearic acid does not have any effect on the quantity of cholesterol.
Effect of amino acids, dopamine and stearic acid on cholesterol production in the liver of rats starved for 72 hours.

(Table Nos. 9a, b; Graph No. 9)

In starving rats, α-aminobutyric acid, arginine, aspartic acid, cysteine, cystine, histidine, hydroxyproline, isoleucine, lysine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine increase the quantity of cholesterol in in vitro studies with the liver tissue. Proline increases the quantity by 100% while isoleucine, serine and valine increase it by 80% and aspartic acid, histidine and threonine do it by only 20%. In case of increase, other amino acids stand in between these two percentages, viz., 100% and 20%. DOPA, glycine and methionine reduce the quantity of cholesterol respectively by 20%, 10% and 10%. Alanine, glutamic acid, leucine and nor-leucine do not have any effect on the quantity of cholesterol, it remains the same as the control. Dopamine and stearic acid also do not have any effect on the quantity of cholesterol.
Effect of amino acids, dopamine and stearic acid on cholesterol production in the spleen of rats starved for 72 hours.

( Table Nos. 10 (a), (b) : Graph No. 10 )

In starving rats, alanine, \( \alpha \)-aminobutyric acid, arginine, aspartic acid, histidine, isoleucine, serine and tryptophan increase the quantity of cholesterol in in vitro studies with the splenic tissue. Alanine increases the quantity by 530% and histidine does the same by only 10%. Other amino acids stand in between these two percentages. Cysteine, DOPA, glutamic acid, glycine, leucine, nor-leucine, lysine, methionine, ornithine, phenylalanine, proline, threonine, tyrosine and valine reduce the quantity of cholesterol in different proportions. Glutamic acid, glycine, leucine, phenylalanine and valine reduce the maximum, that is, 67% while cysteine and methionine reduce it to the minimum that is 17%. Other amino acids stand in between these two percentages. Cysteine does not have any effect on the quantity of cholesterol. It remains the same as the control. Dopamine and stearic acid reduce the quantity by 30% and 67% respectively.
Effect of amino acids, dopamine and stearic acid on cholesterol production in the testis of rats starved for 72 hours.

(Table Nos. 11 (a), (b): Graph No. 11)

In starving rats, $\alpha$-aminobutyric acid, DOPA, glutamic acid, histidine, leucine, ornithine and phenylalanine increase the quantity of cholesterol in in vitro studies with testicular tissue. DOPA, glutamic acid, histidine, leucine, ornithine and phenylalanine increase the quantity by 25% while $\alpha$-aminobutyric acid does the same by 12.5% only. Alanine, arginine, aspartic acid, cysteine, cystine, hydroxyproline, isoleucine, nor-leucine, lysine, methionine, serine, threonine and valine reduce the quantity of cholesterol in different proportions. Lysine and methionine reduce the maximum that is 50% while alanine, cystine, hydroxyproline, isoleucine, nor-leucine and threonine reduce it to the minimum that is 12.5%. Other amino acids stand in between these two percentages. Glycine, proline, tryptophan and tyrosine do not have any effect on the quantity of cholesterol. It remains the same as the control. Dopamine reduces the quantity of cholesterol by 50% while stearic acid reduces it by 12.2% only.
Effect of amino acids, dopamine and stearic acid on cholesterol production in the adrenal of rats starved for 72 hours.

(Table Nos. 12 (a), (b) : Graph No. 12)

In rats starved for 72 hours, alanine and cystine increase the quantity of cholesterol by 100% in in vitro studies with the adrenal tissue. Glutamic acid, histidine, hydroxyproline, leucine, isoleucine, lysine, ornithine, phenylalanine, serine, tryptophan, tyrosine and valine reduce the quantity of cholesterol in different proportions. Histidine, hydroxyproline, leucine, isoleucine, phenylalanine and serine reduce the quantity by 50% and other amino acids reduce it by 25% only. $\alpha$-Aminobutyric acid, arginine, aspartic acid, cysteine, DOPA, glycine, nor-leucine, methionine, proline and threonine do not have any effect on the quantity of cholesterol. Dopamine reduces the quantity by 50% while stearic acid increases it by 100%. 
Effect of amino acids, dopamine and stearic acid on cholesterol production in the liver of rats infected with Cysticercus fasciolaris.

(Table Nos. 13 (a), (b) : Graph No. 13)

In infected rats, \( \alpha \)-aminobutyric acid, arginine, lysine, methionine, proline, serine and tyrosine increase the quantity of cholesterol in \textit{in vitro} studies with liver tissue. Tyrosine increases the quantity by 50\% and arginine and proline do the same by only 8\% while other amino acids stand in between these two percentages.

Alanine, aspartic acid, cysteine, DOPA, histidine, isoleucine, nor-leucine, ornithine and valine reduce the quantity of cholesterol in different proportions. Cysteine reduces the maximum that is 29\% while alanine, DOPA, nor-leucine and valine reduce it to the minimum that is by 9\% only. Other amino acids stand in between these two percentages.

Cystine, glutamic acid, glycine, leucine, phenylalanine, threonine and tryptophan do not have any effect on the quantity of cholesterol. It remains the same as the control. Dopamine in this case increases the quantity of cholesterol but stearic acid does not have any effect on the quantity of cholesterol.
Effect on amino acids, dopamine and stearic acid on cholesterol production in the spleen of rats infected with Cysticercus fasciolaris.

(Table Nos. 14 (a), (b); Graph No. 14)

In infected rats, alanine, \( \alpha \)-aminobutyric acid, arginine, aspartic acid, cystine, DOPA, glutamic acid, leucine, isoleucine and tyrosine increase the quantity of cholesterol in vitro studies with spleen tissue. DOPA increases the quantity by 75% and alanine, \( \alpha \)-aminobutyric acid, aspartic acid, cystine, glutamic acid, leucine, isoleucine and tyrosine do the same by only 25%. Cysteine, nor-leucine, lysine, ornithine, phenylalanine, proline and tryptophan reduce the quantity of cholesterol in different proportions. Lysine reduces the maximum, that is, 75% while nor-leucine, ornithine, proline and tryptophan reduce it to the minimum, that is, 25% only. Other amino acids stand in between these two percentages. Arginine, glycine, histidine, methionine, serine, threonine and valine do not have any effect on the quantity of cholesterol. It remains the same as the control. Dopamine and stearic acid increase the quantity by 25% each.
Effect of amino acids, dopamine and stearic acid on cholesterol production in the testis of rats infected with Cysticercus fasciolaris.

(Table Nos. 15 (a), (b) : Graph No. 15)

In infected rats, $$\alpha$$-aminobutyric acid, arginine, aspartic acid, cysteine, cystine, DOPA, glutamic acid, glycine, histidine, hydroxyproline, leucine, isoleucine, nor-leucine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine increase the quantity of cholesterol in in vitro studies with testicular tissue. Serine increases the quantity by 130% and $$\alpha$$-aminobutyric acid, cystine, glutamic acid, glycine, histidine, hydroxyproline, leucine, isoleucine, nor-leucine, ornithine and phenylalanine do the same by only 25%. Other amino acids stand in between these two percentages. Alanine reduces the quantity of cholesterol by 25%. Lysine and methionine do not have any effect on the quantity of cholesterol and it remains the same as the control. Dopamine and stearic acid increase the quantity of cholesterol by 25% and 100% respectively.
Effect of amino acids, dopamine and stearic acid on cholesterol production in the adrenal of rats infected with Cysticercus fasciolaris.

(Table Nos. 16 (a), (b) : Graph No. 16)

In infected rats, alanine, α-aminobutyric acid, aspartic acid, cystine, DOPA, glutamic acid, lysine, methionine, phenylalanine, proline, serine, threonine and tyrosine increase the quantity of cholesterol in in vitro studies with the adrenal tissue. Cystine increases the quantity by 300% and serine does the same by 104% only. Other amino acids stand in between these two percentages. Cystine, histidine, isoleucine and nor-leucine reduce the quantity of cholesterol. They reduce the quantity by 50%. Arginine, glycine, leucine, ornithine, tryptophan and valine do not have any effect on the quantity of cholesterol. Dopamine and stearic acid increase the quantity of cholesterol by 100% and 210% respectively.
Effect of amino acids, dopamine and stearic acid on cholesterol production in the liver of rats injected with

$\Delta^9$- Tetrahydrocannabinol ($\Delta^9$- THC ).

(Table Nos. 17 (a), (b) : Graph No. 17)

In rats injected with $\Delta^9$- THC, $\alpha$-aminobutyric acid, cystine, DOPA, glutamic acid, histidine, isoleucine, nor-leucine and methionine increase the quantity of cholesterol in in vitro studies with liver tissue. Histidine increases the quantity by 13% and DOPA, glutamic acid, isoleucine, nor-leucine and methionine do the same by only 6%. Other amino acids stand in between these two percentages.

Alanine, arginine, cysteine, lysine, ornithine, phenylalanine, proline, serine, threonine, tyrosine and valine reduce the quantity in different proportions. Alanine and cysteine reduce the maximum, that is 20% while threonine and valine reduce it to the minimum, that is 4%. Other amino acids stand in between these two percentages. Aspartic acid, glycine, leucine and tryptophan do not have any effect on the quantity of cholesterol. It remains the same as the control. Dopamine and stearic acid increase the quantity by 6% and 46% respectively.
Effect of amino acids, dopamine and stearic acid on cholesterol production in the spleen of rats injected with
\[ \Delta^9 - \text{Tetrahydrocannabinol} \ (\Delta^9 - \text{THC}). \]

(Table Nos. 18 (a), (b) : Graph No. 18)

In rats injected with \(\Delta^9 - \text{THC}, \text{DOPA}, \text{histidine, nor-leucine, proline, threonine and tryptophan increase the quantity of cholesterol in in vitro studies with splenic tissue. DOPA increases the quantity by 166\% and histidine and nor-leucine do the same by 16\% only. Other amino acids stand in between these two percentages. Alanine, } \alpha \)-aminobutyric acid, arginine, aspartic acid, glutamic acid, glycine, leucine, methionine, ornithine, phenylalanine, serine, tyrosine and valine reduce the quantity of cholesterol in different proportions. Ornithine reduces the maximum, that is 75\% while alanine and \(\alpha \)-aminobutyric acid reduce it to the minimum, that is 34\% only. Other amino acids stand in between these two percentages. Cysteine, cystine, isoleucine and lysine do not have any effect on the quantity of cholesterol. It remains the same as the control. Dopamine reduces the quantity of cholesterol to 50\% while stearic acid increases it to 25\%.}
Effect of amino acids, dopamine and stearic acid on cholesterol production in the testis of rats injected with
\[ \Delta^9 \text{- Tetrahydrocannabinol (} \Delta^9 \text{- THC) }. \]

(Table Nos. 19 (a), (b) : Graph No. 19)

In the testis of rats injected with \[ \Delta^9 \text{- THC}, \] all amino acids examined and dopamine reduce the quantity of cholesterol in different proportions. It is only the stearic acid that is able to maintain the control amount.
Effect of amino acids, dopamine and stearic acid on cholesterol production in the adrenal of rats injected with

$\Delta^9$-Tetrahydrocannabinol ($\Delta^9$-THC).

( Table Nos. 20 (a), (b) : Graph No. 20 )

In rats injected with $\Delta^9$-Tetrahydrocannabinol, $\alpha$-aminobutyric acid and arginine increase the quantity of cholesterol in in vitro studies with adrenal tissue. Both increase the quantity by 25%. Aspartic acid, cysteine, cystine, DOPA, glutamic acid, glycine, leucine, isoleucine, nor-leucine, methionine, ornithine, phenylalanine, serine, threonine, tryptophan, tyrosine and valine reduce the quantity of cholesterol in different proportions. Aspartic acid reduces the maximum, that is 50% and ornithine, phenylalanine and threonine reduce the minimum that is by 7% only. Other amino acids stand in between these two percentages. Alanine, histidine, lysine and proline do not have any effect on the quantity of cholesterol. It remains the same as the control. Dopamine and stearic acid also do not have any effect on the quantity of cholesterol.
Effect of amino acids, Dopamine and stearic acid on cholesterol production in the testis of rats injected with Cannabidiol (CBD).

(Table Nos. 21 (a), (b) ; Graph No. 21)

In rats injected with Cannabidiol (CBD), nor-leucine, lysine, phenylalanine, proline, serine, threonine and tyrosine increase the quantity of cholesterol in vitro studies with the testicular tissue. Lysine increases the quantity by 83% and nor-leucine and phenylalanine do the same by 4% only. Other amino acids stand in between these two percentages. Alanine, α-aminobutyric acid, arginine, aspartic acid, cysteine, cystine, DOPA, glutamic acid, glycine, leucine, isoleucine, methionine, tryptophan and valine reduce the quantity of cholesterol. α-Aminobutyric acid reduces the maximum, that is, 33% while tryptophan reduces it to the minimum, that is, 4% only. Other amino acids stand in between these two percentages. Histidine and ornithine do not have any effect on the quantity of cholesterol. It remains the same as the control. Dopamine increases the quantity of cholesterol by 33% while stearic acid reduces it by 8%.
Effect of amino acids, dopamine and stearic acid on cholesterol production in the adrenal of rats injected with Cannabidiol (CBD).

(Table Nos. 22 (a), (b) : Graph No. 22)

In rats injected with Cannabidiol (CBD), cysteine, DOPA, leucine, isoleucine, nor-leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tryptophan and tyrosine increase the quantity of cholesterol in *in vitro* studies with the adrenal tissue. Cysteine, isoleucine, lysine and methionine increase the quantity by 75% while DOPA and leucine do the same by 12% only. Other amino acids mentioned above stand in between these two percentages. Alanine, α-aminobutyric acid, arginine, cystine and glutamic acid reduce the quantity of cholesterol in different proportions. α-aminobutyric acid reduces the maximum that is by 50% while cystine reduces it to the minimum that is by 13% only. Other amino acids stand in between these two percentages. Aspartic acid, glycine, histidine and valine do not have any effect on the quantity of cholesterol. It remains the same as the control. Dopamine also does not have any effect on the quantity of cholesterol while stearic acid increases the quantity by 12%.
Effects of the amino acid Alanine on cholesterol production under different conditions in the various organs.

( Table No. 23 )

Alanine is highly cholesterogenic. Except spleen, it shows high production of cholesterol in the liver, adrenal and testis. Maximum values are seen in the adrenal followed by testis. Even under starvation and as also in the infection of cysticercus fasciolaris, response of adrenal is the highest. Spleen is the only other organ which shows 25% increase in the infection. Starvation again induces highest amount of cholesterol in the spleen showing thereby that cholesterol is deposited in the spleen.

$\Delta^9$-THC and CBD reduce the amount of cholesterol in all tissues examined in this study. Thus normal metabolism of alanine is interfered with by the derivatives of Cannabis sativa. Since smoke of "ganja" has a similar effect as that of injections of its derivatives, it may be inferred that probably in human beings also the results will be more or less similar.
Effects of the amino acid, α-aminobutyric acid on cholesterol production under different conditions in the various organs.

(Table No. 24)

α-aminobutyric acid normally increases cholesterol in the testis and liver. There is reduction in the adrenal and the splenic tissue. Under starving conditions, it induces deposition of high quantities of cholesterol in the spleen. Under infection of cysticercus fasciolaris there is uniform increase of cholesterol in all the tissues examined. When the Cannabis derivatives (Δ²-THC or CBD) are injected into the peritoneal cavity, α-aminobutyric acid loses its power of cholesterol production and there is reduction in all tissues except a slight increase in the liver and adrenal.
Effects of the amino acid Arginine on cholesterol production under different conditions in the various organs.

( Table No. 25 )

Except in liver, arginine reduces the quantity of cholesterol in all other tissues examined in this study. There is no change in the adrenal. Starvation has opposite effect except in the case of testis. The high value of cholesterol in the spleen shows that arginine induces its deposition in this organ. Infection of cysticercus fasciolaris has very little effect on the production of cholesterol except in the case of testis where it is increased by 50%.

The cannabis derivatives show interesting results. While Δ⁹-THC increases cholesterol by 25% in the adrenal, CBD decreases it by a similar amount. In other tissues, arginine reduces the amount of cholesterol under the influence of Δ⁹-THC.
Effects of the amino acid Aspartic acid on cholesterol production under different conditions in the various organs.

(Table No. 25)

Aspartic acid is active only in the case of liver. Elsewhere it reduces the quantity of cholesterol. In starvation the effects are variable. Except liver and spleen, there is reduction in the testis and no change in the adrenal. The infection of cysticercus fasciolaris again shows variable results but it induces high amounts of cholesterol in the testis and adrenal through aspartic acid. The cannabis derivatives uniformly reduce cholesterol in all tissues examined in this study. CB1 produces no change in the quantity of cholesterol in adrenal while Δ⁹-THC has no effect on the liver under the influence of aspartic acid.
effects of the amino acid Cysteine on cholesterol production under different conditions in the various organs.

(Table No. 27)

Cysteine increases cholesterol in the adrenal tissue in vitro. In other tissues it is reduced. In starvation also there is uniform reduction except in adrenal where there is no change and in the liver there is slight increase. In the case of infection of cysticercus fasciolaris only the testicular tissue shows a trend towards increase of 75% while all other tissues show decrease under the influence of cysteine. In the rats pretreated with cannabis derivatives there is uniform reduction of cholesterol. CBD however increases cholesterol by 75% in the case of adrenal.
Effects of the amino acid Cystine on cholesterol production under different conditions in the various organs.

(Table No. 28)

Cystine roughly resembles cysteine in its effects on cholesterol contents except in a few cases. Under normal conditions there is 42% increase in cholesterol in the liver tissue and 100% in the adrenal in vitro. In starvation there is a trend towards increase in cholesterol contents except in the testis. In the case of infection of Cysticercus fasciolaris, there is increase of cholesterol in all tissues except in the liver where there is no effect of cystine. In rats pretreated with cannabis derivatives the effects are variable. In adrenal and testis the trend is towards reduction while in the liver and spleen there is slight increase to no change in cholesterol contents.
Effects of the amino acid DOPA on cholesterol production under different conditions in the various organs.

(Table No. 29)

DOPA shows maximum increase of cholesterol in the adrenal. It has no effect on spleen. In the liver it shows an increase of 426 but in the testis there is decrease of about the same magnitude.

In starving conditions, 3:4 dihydroxy phenylalanine (DOPA) shows a plus value only in the testis. In the liver and spleen there is decrease and no change in the adrenal.

Infection of cysticercus fasciolaris brings out a 1006 increase of cholesterol in the adrenal, 756 in the spleen, 506 in the testis and reduction of 96 in the liver under the influence of DOPA.

In the rat pretreated with cannabis derivatives, there is a trend towards increase of cholesterol in the liver and spleen. There is reduction in the adrenal and testis. CDO however, shows a slight increase (126) in the adrenal.
Effects of the amino acid Glutamic acid on cholesterol production under different conditions in various organs.

(Table No. 30)

Glutamic acid shows a high cholesterol increase in adrenal amounting to 100%. In other tissues there is uniformly a decrease. Under starving conditions only testis shows a positive value. However in the infected condition (cysticercus fasciolaris), glutamic acid shows an increase in the amount of cholesterol in the spleen, adrenal and testis. There is no change in the liver. In the pretreated rats injected with cannabis derivatives, glutamic acid reduces the quantity of cholesterol in all tissues except the liver.
Effects of the amino acid Glycine on cholesterol production under different conditions in the various organs.

( Table No. 31 )

Glycine increases the cholesterol contents of the adrenal body but in other tissues there is a marked reduction. In starvation, there is reduction in liver and spleen while there is no change in adrenal and testis. In infected condition (Cysticercus fasciolaris) only the testis shows an increase (23%) while the other tissues (liver, spleen, adrenal) show a decrease in cholesterol contents. In the pretreated rats with cannabis derivatives, there is marked tendency towards reduction in cholesterol contents. Only the liver is static. It shows no change whatever. CBD also produces no change in the adrenal.
Effects of the amino acid histidine on cholesterol production under different conditions in the various organs.

(Table No. 32)

Normally, histidine decreases the quantity of cholesterol in all tissues except adrenal where there is no change. In starvation there is increase in all the tissues examined except the adrenal body. In the infection of Cysticercus fasciolaris, histidine has variable effects. In adrenal and liver there is reduction while there is increase in testis. There is no change from the control in the spleen.

In the rats pretreated with cannabis derivatives histidine again shows variable effects. In liver and spleen there is increase in the amount of cholesterol while in other tissues there is reduction or no change with $\Delta^9$-THC. CBD has no effect on testis and adrenal under the influence of histidine.
Effects of the amino acid Leucine on cholesterol production under different conditions in the various organs.

(Table No. 33)

Leucine is ketogenic amino acid and hence it is expected that it will increase the amount of cholesterol. However, it is only in the case of adrenal there is enhancement of cholesterol. In other tissues there is reduction. In starvation there is increase only in the case of testis. Similar is the condition in infected (cysticercus fasciolaris) rats. The increase is seen only in the testis and spleen. In $^{14}$C-treated rats there is uniform reduction in practically all tissues. CBD however increases cholesterol to the extent of 126 in the adrenal.
Effects of the amino acid Isoleucine on cholesterol production under different conditions in the various organs.

(Table No. 34)

Isoleucine has a very great influence on the cholesterol production in the adrenal. The quantity of cholesterol increases three times in the adrenal while elsewhere it is reduced. In testis there is no change. In starving conditions, increase is seen in the liver and spleen while in the adrenal and testis there is reduction. In the infected condition (cysticercus fasciolaris) the results are variable. The testis shows 25% increase while in other tissues there is reduction. Spleen shows no change.

In the rats pretreated with cannabis derivatives there is slight increase in the liver. In other tissues there is reduction in the amount of cholesterol. CBD however is remarkable in producing a 75% increase in the adrenal under the influence of isoleucine.
Effects of the amino acid nor-leucine on cholesterol production under different conditions in various organs.

(Table No. 35)

Like leucine and isoleucine, nor-leucine shows greatest activity in the adrenal. In spleen there is reduction and in other tissues there is practically little or no change. In starvation again there is reduction in the quantity of cholesterol in the spleen by half. Liver and adrenal show no change while in testis quantity is reduced by 13\%.

Infection with cysticercus fasciolaris brings about an increase by 25\% in the testis. All other tissues show reduction.

In the rats pretreated with $\Delta^9$-THC there is reduction in all tissues except the liver where there is slight increase. CBD brings about an increase of 4\% in the testis and 50\% in the adrenal under the influence of nor-leucine.
Effects of the amino acid Lysine on cholesterol production under different conditions in the various organs.

( Table No. 36 )

Lysine uniformly reduces the quantity of cholesterol in all the tissues examined. The maximum reduction is 90% in the adrenal. In starvation the results are variable depending upon the tissue concerned e.g. it is plus 40% in liver and minus 75% in the adrenal.

In the infection of cysticercus fasciolaris there is increase in the liver and adrenal while in other tissues there is reduction or no change.

In the rats injected with $\Delta^9$-THC, there is no change in the spleen and adrenal elsewhere there is slight reduction. C3H brings about an increase in the adrenal and testis under the influence of lysine.
Effects of the amino acid Methionine on cholesterol production under different conditions in the various organs.

(Table No. 37)

The methionine does not affect the quantity of cholesterol in the liver, adrenal and testis under normal conditions. In starvation there is reduction. In the infection of cysticercus fasciolaris there is increase only in the adrenal and liver. Under the influence of $\Delta^9$-THC, methionine brings about reduction in all tissues examined except in the liver.

In spleen there is reduction in all conditions except in the infected condition where there is no change.

In the adrenal, the conditions are variable. There is no change under normal and starvation conditions but there is increase in the quantity of cholesterol in infected conditions. Injected rats ($\Delta^9$-THC) show reduction by 27%. CBD increases cholesterol in the adrenal by 75% and reduces it in the testis by 17%.
Effects of the amino acid Ornithine on cholesterol production under different conditions in the various organs.

(Table No. 38)

In normal rats, ornithine enhances the quantity of cholesterol specially in the adrenal and testis. The increase is 100% in the adrenal and only 33% in the testis. Under starvation there is increase in the liver and testis and reduction in the spleen and adrenal. Infection of cysticercus fasciolaris brings about an increase in the quantity of cholesterol under the influence of ornithine only in the testis.

The rats treated with injections of Δ⁹-THC, a decrease in the quantity of cholesterol in all organs examined. CBD (cannabidiol) produces no change in the testis but raises cholesterol by 37% in the adrenal.
Effects of the amino acid phenylalanine on cholesterol production under different conditions in the various organs.

(Table No. 39)

Phenylalanine increases the quantity of cholesterol by 400% in the adrenal of normal rats. This is perhaps the highest amount among all the other amino acids examined. In other organs there is reduction in the liver and testis but there is slight increase in the spleen. Under conditions of starvation, on the contrary, the quantity of cholesterol is increased by 50% in the liver and 25% in the testis. In spleen and the adrenal the quantity is reduced by about one half.

In the rats infected with cysticercus fasciolaris, there is increase of cholesterol due to phenylalanine in the adrenal and testis, while there is reduction in the spleen and no change in the liver. There is uniform reduction of cholesterol in all tissues examined in rats pretreated with $A^9$-THC. CBD however, shows a slight increase in the testis and adrenal.
Effects on the amino acid Proline on cholesterol production under different conditions in the various organs.

(Table No. 40)

Under normal conditions proline reduces the quantity of cholesterol in all tissues except the adrenal where there is no change. During starvation it is in the liver that the increase is the maximum. In the infection of cysticercus fasciolaris the maximum increase is in the adrenal and slight increase in the liver and 30% in the testis. \(^9\)THC and proline induce maximum deposition of cholesterol in the spleen (1336) elsewhere there is reduction. 930 produces 12% increase in the cholesterol content of the testis and 25% in the adrenal.
Effects of the amino acid Serine on cholesterol production under different conditions in the various organs.

( Table No. 41 )

Serine increases the amount of cholesterol in the testis and adrenal. In the adrenal the increase is maximum (100%). In starvation, the increase is seen in the liver and spleen only. Infection of cysticercus fasciolaris brings about increase of cholesterol in all organs examined except the spleen. Under the influence of \( \Delta^9 \)-THC and serine there is uniformly reduction in the quantity of cholesterol in all organs. CBD injections and serine, however, bring about slight increase in the testis and adrenal.
Effects of the amino acid Threonine on cholesterol production under different conditions in the various organs.

(Table No. 42)

Threonine reduces the amount of cholesterol in the liver, spleen and the testis in varying proportions. There is no change in the adrenal. In starvation there is slight increase in the liver while in other organs there is no change or slight reduction.

Infection of cysticercus fasciolaris induces slight increase (10%) in the adrenal and testis (50%) under the influence of threonine. In other organs there is no change.

Injections of $\Delta^9$-THC reduce the amount of cholesterol in all organs except the spleen where there is extra deposition of cholesterol. Injections of CBD increase the amount of cholesterol in the testis and adrenal.
Effects of the amino acid Tryptophan on cholesterol production under different conditions in the various organs.

(Table No. 43)

Under normal conditions, tryptophan reduces the amount of cholesterol in all organs examined except in the adrenal where there is no change. In starving conditions tryptophan produces no change in the testis but in the liver and spleen there is increase in the amount of cholesterol. In adrenal there is reduction. Infection of cysticercus fasciolaris induces 100% increase in the testis. In the rats pretreated with $\Delta^9$-THC there is increase of cholesterol in the spleen. In other organs there is reduction or no change. CBD injections and tryptophan bring about reduction of cholesterol in the testis and increase by one half in the adrenal.
Effects of the amino acid Tyrosine on cholesterol production under different conditions in the various organs.

(Table No. 44)

Tyrosine increases the amount of cholesterol in the testis under normal conditions. In starvation increase is seen only in the liver. In the infection of cysticercus fasciolaris there is increase in the amount of cholesterol through the agency of tyrosine in all the organs examined. Under the influence of $\Delta^2$-THC and tyrosine there is uniformly reduction in the amount of cholesterol in the liver, spleen, adrenal and the testis. CBD and tyrosine increase cholesterol in the testis and adrenal.
Effects of amino acid Valine on cholesterol production under different conditions in the various organs.

( Table No. 45 )

Under normal conditions valine increases the quantity of cholesterol in the adrenal and spleen. In starvation cholesterol is seen deposited in the liver. In other organs it is reduced. In the infected condition there is increase only in the testis. Other organs show no change or only slight reduction. Injections of \( \Delta^9 \)-THC bring about reduction in the cholesterol contents in all organs under the influence of valine. CBD and valine also reduce the amount of cholesterol in the testis and have no effect on the adrenal.
Effects of Dopamine on cholesterol production under different conditions in the various organs.

(Table No. 46)

Dopamine reduces the amount of cholesterol only in the liver. In other organs there is increase. Maximum increase is seen in the adrenal (50%). Starvation uniformly reduces the amount of cholesterol in all organs except in the liver where there is no change. Infection of cysticercus fasciolaris induces the increase in the amount of cholesterol in all organs examined, the maximum being in the adrenal (100%).

In the rats pretreated with injections of $\Delta^9$-THC spleen shows reduction in the amount of cholesterol by one half. In the liver there is slight increase while there is no change in the adrenal. In the testis there is reduction. CBD injections increase the amount of cholesterol in the testis by one third and produce no change in the adrenal.
Effects of Stearic acid on cholesterol production under different conditions in the various organs.

(Table No. 47)

Stearic acid under normal conditions produces more of cholesterol in the spleen. In other organs (liver and the testis) there is slight reduction. Under starvation there is increase in the amount of cholesterol only in the adrenal. In the rats infected with the cysticercus fasciolaris, there is increase in the amount of cholesterol in the adrenal (210%) and the testis (100%).

In the rats pretreated with $\Delta^9$-THC and under the influence of stearic acid, liver and spleen show increase in the amount of cholesterol while the adrenal and the testis show no change. CBD injections and stearic acid reduce the amount of cholesterol in the testis but increase it in the adrenal.
Biosynthesis of Cholesterol

Many experiments have been performed to find out the compounds involved in cholesterol synthesis since Dezani (1913) who pointed out an increase in the amount of cholesterol in rats maintained on a diet without cholesterol (Kritchevsky, 1958). Rittenberg and Schoenheimer (1937) utilized deuterium in water. Half the isotope was detected in cholesterol of experimental mice.

Sonderhoff and Thomas (1937), MacLeod and Smedley-MacLean (1938) and finally Bloch and Rittenberg (1942) came to the conclusion that acetate is utilised in cholesterol synthesis. Since then pyruvate (Anker, 1948; Anker and Raper, 1948), acetamide (Anker and Raper, 1948), acetaldehyde (Brady and Gurin, 1951), ethanol (Bloch and Rittenberg, 1944), acetone (Price and Rittenberg, 1930), butyrate (Brady and Gurin, 1950), valerate (Bloch and Rittenberg, 1944), hexanoate (Brady and Gurin, 1950), octanoate (Brady and Gurin, 1950), and palmitate (Bloch and Rittenberg, 1944) have been shown to be converted to cholesterol. Butyrate, isovalerate and leucine also stand prominently as precursors of cholesterol (Bloch, 1944). The ability of the above compounds has been traced to the formation of active acetate or acetoacetate. Steps in the synthesis were worked out by Cornforth (1939) which show the sequence of events as Acetyl CoA $\rightarrow$ Acetoacetyl CoA $\rightarrow$ Hydroxy-β-methyl-glutaryl-CoA $\rightarrow$ Mevalonic acid $\rightarrow$ Mevalonyl pyrophosphate $\rightarrow$ Isopentenyl pyrophosphate $\rightarrow$ Farnesy 1,4-diphosphate $\rightarrow$ Squalene $\rightarrow$ Lanosterol $\rightarrow$ Cholesterol.
Biosynthesis of cholesterol from amino acids

The behaviour of amino acids may be considered from the point of view of metabolic end products they produce. Amino acids that form pyruvic acid or a member of the citric acid cycle convertible into pyruvic acid and produce glucose and glycogen are said to be glucogenic or glycogenic. The amino acids that yield acetyl CoA or acetoacetic acid and increase the excretion of ketone bodies in diabetic animals are said to be ketogenic. Some amino acids such as phenylalanine, tyrosine and isoleucine yield precursors of both ketone bodies and glucose and thus are both ketogenic and glucogenic. Most of the amino acids are glucogenic. Leucine is purely ketogenic. The amino acids that stimulate cholesterol production do not belong to any one of the above categories. Further the behaviour of amino acids also differs in the various organs under different conditions as regards production of cholesterol. There is thus the necessity of the study of the complete metabolism in different organs. Since pyruvic acid can be converted to acetyl CoA through a series of complex reactions which require lipoic acid, nicotinamide adenine dinucleotide phosphate and thiamine pyrophosphate (Reed, 1957; Soike and Reed, 1959; Goldman and Vagelos, 1954), the amino acids that produce pyruvic acid can also produce cholesterol. Acetyl CoA is the starting point for the biosynthesis of cholesterol. The known metabolic pathways leading to the formation of pyruvic acid/acetyl CoA in the mammalian tissues are as follows:
1. Glycine:

Although glycine is classified as glucogenic, it can very easily influence the synthesis of cholesterol. Glycine is oxidised through reaction with acetyl CoA to amino acetone (Urata and Granick, 1953). Amino-acetone can be converted to methyl glyoxal lactic acid, pyruvic acid and finally to acetyl CoA. In the formation of methyl glyoxal, CoASH is recovered as such and can be utilized again. Formation of amino acetone is a common process in the liver through the enzyme, amino acetone synthetase. According to Granick (1953) about one-fourth of glycine can undergo change to amino acetone. If the pyruvic acid ultimately formed in the reactions could be converted to acetyl CoA, it can affect the biosynthesis of cholesterol by 25%. Glycine can also be converted to serine.

Influence of Glycine on cholesterol formation
2. Serine:

Apart from interconvertibility with glycine, serine is deaminated to the pyruvic acid by the action of serine dehydrase. This process accounts in part for the glucogenic nature of serine.

\[
\begin{align*}
\text{CH}_2 - \text{CH} (\text{-H}_2\text{O}) & \quad \text{CH}_2 - \text{CH}_3 (\text{+H}_2\text{O}) & \quad \text{CH}_3 + \text{H}_3 \\
\text{HC} - \text{NH}_2 & \quad \text{C-NH}_2 & \quad \text{C} = \text{NH-Mg}^{++} & \quad \text{C-OH} \\
\text{COOH} & \quad \text{COOH} & \quad \text{COOH} & \quad \text{COOH}
\end{align*}
\]

The enzyme L-serine dehydrase in liver brings about this reaction with pyridoxal phosphate as co-enzyme (Jayne and Greenberg, 1955). High protein diet and diabetic conditions increase the activity of this enzyme seven to eight times (Freeland and Avery, 1964).

3. Alanine:

The amino group is removed primarily by transamination, but possibly to some extent by an amino acid oxidase, resulting in the formation of pyruvate; it is therefore glucogenic (according to Hawk, 1975-77). This amino acid can undergo both deamination by \(\alpha\)-amino oxidase and transamination with \(\alpha\)-ketoglutaric acid. The net result is the formation of pyruvic acid (Haugen, 1947; Meister, 1937).
Thus metabolism of alanine can be put down as follows:

```
Glucose
    ↑
Alanine → pyruvic acid
    ↓
acetoacetic acid → Acetyl CoA
    ↓
Cholesterol
```

4. Threonine:

Threonine does not undergo transamination. It is catabolized by two pathways, both of which lead to two products that can be converted to glucose, it is therefore potentially glucogenic (Hawk, 1975-77). Apart from splitting into glycine and acetaldehyde,
threonine forms amino acetone through dehydrogenation and spontaneous decarboxylation (Drata and Granick, 1953; Neuberger and Tait, 1953). Influence of amino acetone in cholesterol formation has already been discussed along with the metabolism of glycine. Overall reactions are as follows:

\[
\begin{align*}
\text{\textit{\alpha}-Amino} & \quad \longrightarrow \quad \text{Threonine} & \quad \longrightarrow \quad \text{Glycine} & \quad + \quad \text{Acetaldehyde} \\
\text{\textit{\beta}-Keto} & \quad \text{butyric acid} & \quad \longrightarrow \quad \text{\textit{\alpha}-Ketobutyric acid} \\
\text{Amino acetone} & \quad \longrightarrow \quad \text{\textit{\alpha}-Aminobutyric acid} \\
\text{Methylglyoxal} & \quad \longrightarrow \quad \text{Pyruvic acid}
\end{align*}
\]

5. Valine:

Catabolism of valine leads to propionyl CoA or succinyl CoA; it is therefore glucogenic (Hawk, 1975-77). Studies of Coon et al (1953), Weister (1957) and Kreb (1964) have shown that pyruvic acid is the end product in the metabolic pathway of valine. Transamination leads to the formation of \textit{\alpha}-ketoisovaleric acid which condenses with acetyl CoA to form isobutyryl CoA. Other intermediates are methylacrylyl CoA, \textit{\beta}-hydroxyisobutyric acid, methylmalonic semialdehyde, methylmalonyl CoA and propionyl CoA. Methylmalonyl CoA can be interconverted to succinyl CoA by an isomerase. Succinyl CoA can end up in succinic acid and finally into pyruvic acid.
5. Leucine:

Catabolism of leucine leads to acetoacetic acid and acetyl CoA, it is therefore ketogenic. It has been found to be the most ketogenic of all the amino acids (Hawk, 1976-77). Through transamination it forms \( \alpha \)-Ketoisocaproic acid and through intermediates isovaleryl CoA, \( \beta \)-methylcrotonyl CoA, \( \beta \)-methylglutaconyl CoA and \( \beta \)-hydroxy-\( \beta \)-methyl glutaryl CoA and finally ends up in the acetoacetic acid and acetyl CoA. All these products are key compounds in the biosynthesis of cholesterol (Meister, 1957; Krebs, 1964). One mol of leucine can yield 1.5 mols of acetoacetic acid.

7. Isoleucine:

Reactions analogous to those seen in the metabolic breakdown of valine and leucine are also seen in the case of isoleucine (Meister, 1957; Krebs, 1964). Transamination leads to the formation of \( \alpha \)-keto-\( \beta \)-methyl valeric acid which, through intermediates, \( \alpha \)-methylbutyryl CoA, \( \alpha \)-methylcrotonyl CoA, \( \alpha \)-methyl -\( \beta \)-hydroxybutyryl CoA and \( \alpha \)-methylacetoacetyl CoA finally ends up in the propionyl CoA and acetyl CoA. Isoleucine is, therefore, both ketogenic and glucogenic.

8. Lysine:

This essential amino acid undergoes deamination to \( \alpha \)-keto-\( \epsilon \)-amino caproic acid. Stern and Co-workers (1960) and
Rithstein, Cooksey and Greenberg (1962) have shown that the final product acetoacetyl CoA is obtained through a number of intermediates like $\Delta^1$-piperidine - 2 - carboxylic acid, piperolic acid, $\Delta^3$-piperidine - 2 - carboxylic acid, $\alpha$-amino-adipic acid - E-semialdehyde, L-$\alpha$-amino adipic acid, $\gamma$-keto-adipic acid, glutaryl CoA and crotonyl CoA. Acetoacetyl CoA can directly enter in the biosynthesis of cholesterol. Lysine can be converted to $\gamma$-ketoglutaric acid, thus it is potentially glucogenic.

9 - 10 Aspartic and Glutamic acids:

Both are glucogenic. The amino group of aspartic acid may be removed by transamination to form oxalacetic acid and it is therefore glucogenic (Hawk, 1976-77). Both are very reactive amino acids. Through transamination reactions they can influence citric acid cycle. Reverse reactions of citric acid cycle intermediates can lead to the formation of pyruvic acid. These amino acids and their derivatives (N-acetyl-L-aspartic acid, $\gamma$-amino butyric acid etc.) are found in the brain in large amounts.

They can influence the biosynthesis of cholesterol mainly, indirectly through various reactions.

Both acids yield glucose in diabetic animals and in each case three carbons are utilized. Pyruvic acid thus, is formed which may as well undergo reactions to get converted into acetyl
CoA, instead of into glucose. Glutamic acid and aspartic acid and their derivatives are also end products in the metabolism of other amino acids like proline, hydroxyproline, arginine and ornithine.

11 - 12 - 13  Phenylalanine, Tyrosine and DOPA

The metabolism of these amino acids forms a series which ends ultimately in the production of acetoacetic acid and fumaric acid. These amino acids are both ketogenic and glucogenic. Phenylalanine is irreversibly hydroxylated to tyrosine. Tyrosine is converted to p-hydroxyphenylpyruvic acid (Kenney, 1959) which is oxidised to homogentisic acid. Details of the process have been worked out by Weinhouse and Millington (1951), Lerner (1953), Uchida et al (1954), Kenney (1959) and Cannoni and La Du (1959). Process can take place even under anaerobic conditions as shown by Shrivastava (1975) through the effect of keto-enol tautomerization of the phenylpyruvates. Homogentisic acid is normally oxidised to maleylacetoacetic acid which is isomerized to fumarylacetoacetic acid and broken into fumaric acid and acetoacetic acid through the hydrolase. Acetoacetic acid can be directly utilized in the biosynthesis of cholesterol after combining with coenzyme A.

14  Tryptophan :

This essential amino acid is L-α-amino-β-indole propionic acid. According to Holson et al (1952) mammalian liver is capable
of oxidation of the benzene ring of tryptophan and finally end up in acetyl CoA. The intermediates according to them are N-Formylkynurenine, Kynurenine, 3-Hydroxykynurenine, 3-Hydroxyanthranilic acid, \( \alpha \)-Aroyl-3-amino fumaric acid, \( \gamma \)-Oxalocrotonic acid, \( \gamma \)-Ketoadipic acid, Glutaryl CoA and finally acetyl CoA. It is interesting to note that three carbons of tryptophan can give rise to alanine through separation of side chain of kynurenine and 3-hydroxykynurenine. Alanine in its turn can form pyruvic acid. It is very shortly glucogenic (Hawk, 1973-77).

15. Histidine : (\( \alpha \)-amino-\( \beta \)-imidazole-propionic acid)  

The main reaction which can influence the cholesterol synthesis is the transamination (Spalter and Baldridge, 1963).

Histidine + pyruvate \( \rightarrow \) Imidazole pyruvate + alanine.

Another pathway results in the release of glutamic acid (Borek et al., 1955; Haenselens and Osborn, 1959). Thus alanine and glutamic acids formed during catabolism can influence cholesterol formation. This amino acid is glucogenic.

16. Arginine (\( \alpha \)-amino-\( \gamma \)-guanidovaleric acid) :

Glucogenic.

17. Ornithine (\( \gamma \)-diaminovaleric acid) :

Glucogenic.

18. Proline :

Glucogenic.
19. Hydroxyproline

Glutamic acid is the end product in the metabolism of these amino acids (Coon and Robinson, 1950; Reisach and Strecker, 1952; Stetten and Schoenheimer, 1951). Hydroxyproline is converted to γ-hydroxy glutamic acid by rat liver enzymes.

The remarkable intermediates in the metabolism of hydroxyproline, so far as the present discussion is concerned, is γ-hydroxy-β-ketoglutaric acid (Goldstone and Adams, 1962). The steps are as follows:

\[
\begin{align*}
\text{γ-Hydroxy-\text{acid}} & \quad \text{Oxalacetic acid} & \quad \text{Aspartic acid} \\
\text{γ-Ketoglutaric acid} & \quad \text{Glyoxyl acid} & \quad \text{Lactic and Pyruvic acid}
\end{align*}
\]
Alanine may also be formed if transamination takes place with pyruvic acid, glyoxyllic acid may form glycine by transamination with glutamine, ornithine and others. The interrelationship may be expressed as follows:

\[
\text{Proline} \quad \text{Hydroxyproline} \quad \text{Alanine} \quad \rightarrow \quad \text{Pyruvic acid}
\]

\[
\text{Glutamic acid} \quad \text{Pyruvic acid}
\]

\[
\text{Proline} \quad \text{Ornithine} \quad \text{Arginine}
\]

Since pyruvic acid can be derived from these amino acids, either glucose or acetyl CoA can be formed. Acetyl CoA may start the production of cholesterol.

20 - 21 - 22 Methionine, Cystine and Cysteine

Cystine can be derived from methionine. Transamination of methionine with \(\alpha\)-ketoglutaric acid leads to the formation of \(\alpha\)-keto-\(\gamma\)-methylthiobutyric acid and glutamic acid (Weister, 1957). In the formation of cysteine (Cystine) only sulphur of methionine is utilized, rest of the cysteine molecule is derived from serine. Remaining part of methionine forms homoserine which ultimately gives rise to \(\alpha\)-ketobutyric acid \(\rightarrow\) propionic acid. Propionic acid is converted to succinic acid or succinyl CoA through methylmalonyl CoA. Succinic acid can break into pyruvic acid or enter the citric acid cycle. These steps are seen in the case of valine also.
The effective metabolic products then are glutamic acid, succinic acid and pyruvic acid.

Cysteine can form pyruvic acid / glutamic acid in several ways in different organs (Tarr, 1933).

(i)

\[
\text{H} \quad \text{desulphydrase} \quad \text{NH}_2
\]

\[
\text{SH} - \text{CH}_2 - \text{C} - \text{SO}_2\text{H} \quad \longrightarrow \quad \text{H}_2\text{S} - \text{CH}_2 - \text{C} - \text{SO}_2\text{H} \quad \text{pyridoxal phosphate} \quad \text{NH}_2
\]

Cysteine \quad \text{CH}_3 - \text{C} - \text{SO}_2\text{H}

imino acid

\[+ \quad \text{H}_2\text{O} \quad \text{CH}_3 - \text{CO} - \text{COOH} + \text{NH}_3\]

pyruvic acid

(ii) Transamination with \(\alpha\)-ketoglutaric acid gives rise to \(\delta\)-mercapto-pyruvic acid and glutamic acid.

(iii) \(\delta\)-mercapto-pyruvic acid is broken into pyruvic acid and sulphur in animal tissues.
\[
\text{HS - CH}_2 - \text{CO - COOH} \rightarrow \text{CH}_3 - \text{CO - COOH} + S
\]

In short, the interrelationship for the present discussion can be expressed as follows:

\[
\text{Homocysteine} \\
\text{Methionine} \quad \downarrow \quad \rightarrow \quad \gamma\text{-Ketobutyric} \quad \rightarrow \quad \text{Propionic acid}
\]

\[
\text{Sulphur} \quad \text{Homoserine} \quad \downarrow \\
+ \quad \text{Serine} \quad \text{Pyruvic acid} \quad \text{Succinic acid} \\
\downarrow \\
\text{Cysteine} + \text{NH}_3 + \text{H}_2\text{S} \quad \text{Pyruvic acid}
\]

\[
\text{Cysteine sulphinic acid} + \text{Pyruvic acid} + \text{SO}_2
\]

\[
\beta\text{-Mercaptopropionic acid} \rightarrow \text{Pyruvic acid} + S
\]

\(
\beta\text{-Mercaptopropionic acid} \rightarrow \text{Pyruvic acid} + S
\)
Cholesterol and neutral 17-ketosteroids

Dahia (1977) and Dahia and Shrivastava (1978) have examined the metabolism of neutral 17-ketosteroids in rats in starvation and under the influence of $\Delta^9$-THC. The ketosteroids form a class of compounds in which the cyclopenta[ceph]phenanthrene ring has a ketone ring in the position 17. They are metabolic products of adrenal and gonadal steroids. Since these steroids are derived from cholesterol, it is worthwhile to compare the findings of the above workers with those of the present study. The urinary ketosteroids are reduced in starvation as also in the rats treated with $\Delta^9$-THC. The cholesterol and the neutral 17-ketosteroids contents vary as follows in the different conditions.

Table No. 62

Change in the average values of the cholesterol and the neutral 17-ketosteroids contents under starvation for 72 hours.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Cholesterol + or - % change (mg/ml)</th>
<th>Ketosteroids + or - % change (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>+ 5.560 %</td>
<td>- 35.725 %</td>
</tr>
<tr>
<td>Spleen</td>
<td>+ 271.50 %</td>
<td>- 1.751 %</td>
</tr>
<tr>
<td>Testis</td>
<td>+ 75.12 %</td>
<td>- 1.316 %</td>
</tr>
<tr>
<td>Adrenal</td>
<td>+ 486.55 %</td>
<td>- 1545.717 %</td>
</tr>
</tbody>
</table>
Table No. 63

Change in the average values of cholesterol and the neutral 17-Ketosteroids contents under the influence of Δ⁹-THC.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Cholesterol</th>
<th>Ketosteroids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ or - % change</td>
<td>+ or - % change</td>
</tr>
<tr>
<td></td>
<td>(mg % ml )</td>
<td>(mg % ml )</td>
</tr>
<tr>
<td>Liver</td>
<td>+ 394.56 %</td>
<td>- 15.413 %</td>
</tr>
<tr>
<td>Spleen</td>
<td>- 375.00 %</td>
<td>+ 41.774 %</td>
</tr>
<tr>
<td>Testis</td>
<td>+ 129.81 %</td>
<td>+ 53.480 %</td>
</tr>
<tr>
<td>Adrenal</td>
<td>+ 4320.00 %</td>
<td>- 1127.273 %</td>
</tr>
</tbody>
</table>

In starvation there is uniform increase of cholesterol in all organs and decrease in the ketosteroids showing thereby that there has been no conversion of all the available cholesterol into steroids and that the metabolism of steroids themselves has been reduced. On the other hand the conditions are different under the influence of Δ⁹-THC. In the testis there is not only increase in the cholesterol but also in the neutral 17-Ketosteroids. The metabolic activity of the testis is thus increased and will lead to greater sexual activity. Although enhanced sexual activity is indicated by the present study, there is muscular dystrophy in mice after chronic subcutaneous treatment with Cannabinoids (Giusti et al., 1977).
The collective influence of amino acids on the quantity of cholesterol has been studied by statistical studies of correlation and regression. The amino acids decrease the cholesterol in the liver, spleen, testis and adrenal after starvation for 72 hours. The greatest decrease is in the spleen which is, on the basis of regression studies, of the order of 324.32%. In the liver, it is 24% while in the testis and adrenal it is about 10%.

In the rats infected with Cysticercus fasciolaris, the amino acids increased the cholesterol in the spleen and testis while it is reduced in the liver and adrenal. The maximum increase is in the spleen (17.07%). The decrease is 12.45% in the liver. It is very little (0.3%) in the adrenal.

When injections of $\Delta^9$-THC are administered to the rats, the collective influence of the amino acids is to increase the amount of cholesterol in the liver and testis. The increase is by about one-third in the testis and 3.71% in the liver on the basis of regression studies. In the spleen and adrenal, the amino acids reduce the quantity of cholesterol under the influence of $\Delta^9$-THC. In the spleen, the reduction is 31.57% while in the adrenal it is 20%.

The effect of Cannabidiol (CBD) on the behaviour of
amino acids as regards production of cholesterol is opposite to that of $\Delta^9$-THC. In the testis, the amino acids reduce the quantity of cholesterol and increase it in the adrenal. The reduction in the testis is of the order of 19.92 % and the increase is 15 % in the adrenal. The net result of the collective activity of the amino acids is mainly to bring about the normal equilibrium for example, in starvation the cholesterol is increased in all the organs examined while amino acids collectively reduce it in varying proportions. There are only a few exceptions to this antagonistic behaviour as seen from the following table:

**Table No. 64**

Collective effect of amino acids on:
(a) Normal increase (+) or decrease (-)
(b) Collective effect of amino acids.

<table>
<thead>
<tr>
<th></th>
<th>Starvation</th>
<th>Infection of Cysticercus fasciolaris</th>
<th>$\Delta^9$-THC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liver</strong></td>
<td>a</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>Spleen</strong></td>
<td>a</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Testis</strong></td>
<td>a</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>Adrenal</strong></td>
<td>a</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>